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CERTAIN ABNORMALITIES IN MILLETS INDUCED BY X-RAYS

By N. Krishnaswami, B.Sc., Ph.D. (Kiel)
AND
G. N. Rangaswami Ayyangar, F.N.I., I.A.S.
(Agricultural Research Institute, Coimbatore)

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Irradiation with X-rays has been much resorted to by many workers in genetics and plant breeding in order to induce genetic variability. It has been the common experience of almost all the workers that in the first generation, are produced a number of mutations which are usually recessives and at the same time many of them may take the shape of abnormalities rarely met with in natural populations. Mutations more staid and of economic value have been obtained in the later generations. This method was used for the millets also and a few abnormalities were noted in the progenies of the X-rayed seeds of *Eleusine coracana* Gaertn. and *Pennisetum typhoides* S. & H. Since these abnormalities are the first of their kind reported in these millets a short account of them is given below.

I. E. coracana (*Ragi*)

The technique employed in the X-raying of the seeds of ragi (E.C. 593—Green—throughout, top curved, brown grained) was the same as for *P. typhoides* (Krishnaswamy and Ayyangar, 1941). The germination of the exposed seeds was slow and irregular. Many seedlings died even before the protrusion of the seedling leaves. A number of albino seedlings occurred. These were slightly yellower than the usual albinos met with in the normal populations. The incidence of the albinos and the number of survivors were as follows:

Treatments	No. of seeds	No. of albinos	No. of survivors
A. 1 hour	200	38	84
B. 2 hours	200	18	7
C. 3 ,, ...	200	..	..
Control	200	..	170

Thus as in the case of *P. typhoideum*, here also two hours exposure proved to be just sublethal and three hours lethal. Some of the albinos in treatment

A, later produced a green shoot and grew into mature normal plants. Out of the 91 X_1 plants 9 were semi-sterile and 4 had gappy panicles (Krishnaswamy, 1939). The gappiness in two plants was nearly 2 in. in length.

(*a*) *White banded leaves* (Fig. 1):

In two cultures of the X_2 generation some of the seedlings showed an irregular albino zone in the leaves. When the seedlings were only two leaved the latter showed constrictions of the margins at the albino zones. The next three leaves showed definite green and white zones alternating. The white bands ran in patches across the whole width of the blade. The mature leaves, however, were completely green. In the X_2 generation the total number of normal to banded seedlings was 483 greens to 148 banded. In the succeeding generations no white banded seedlings appeared in the progenies of a number of green selections, while they appeared in the progenies of all the white banded selections (total of 5 families Pure green—784: white banded 1443).

White banded seedlings (Zebra) have been reported in Maize (Eyster, 1934). Four kinds of Zebra-striping zb_1, zb_2, zb_3 and zb_4 are described. The banding in ragi is similar to the zb_2 and zb_4, manifesting in the seedling stages and disappearing in the mature plants. In Maize they are simple recessives. In the progenies of an artificial cross in *Rice*, Ramiah and Ramanujam (1936) found similar striped seedlings. In this case, however, the striped seedlings bred pure and behaved as simple recessives. Cross banded seedlings have occurred in the natural populations of *Sorghum sudanense* (Ayyangar and Ponnaiya, 1939), inherited as simple recessives.

Albinism has been reported in Ragi (Ayyangar and Krishna Rao, 1931) as caused by a pair of duplicate factors. In these segregations, however, the heterozygous dominants are not distinguishable from the homozygous one, *i.e.*, normal green and the homozygous recessives lived till the reserve food material in the seeds was exhausted. In the present case the white banded seedlings have always segregated into green and white banded seedlings, while the greens have bred true. This behaviour is obviously due to the heterozygosity of the white banded seedlings. The banded seedlings come to flower a week later than the homozygous greens probably as a result of heterosis. The inheritance of this character is being further followed.

(*b*) *Foliation of the panicle* (Figs. 2 and 3):

In another culture out of 374 plants, 8 were noted to have produced no panicles, while the rest had them. Instead of the normal panicle a leafy bunch appeared in these eight plants. Except for this the plants were not

vegetatively in any way different from the others. These abnormal panicles were borne on five main rachii corresponding to the five spikes. In the individual bunches each was seen to have five leafy structures corresponding to the two glumes and three lemmas. Of course only a few basal spikelets had developed into such leafy growths, while the rest had been suppressed. When examined for any pathogenic organism, it was found to be quite normal and healthy.

These bunches were found producing roots and when transplanted each grew into a large plant and again produced the abnormal panicles. The foliated panicles tended to become shorter in length and those formed on the secondary and tertiary branches, especially showed tendencies of reversion to the normal glume-like appearance. In some extreme cases of reversion to original type the panicles had become stumpy with almost normal glumes. In these the flowers contained much reduced ovary and stamens.

Similar foliations of the entire or a portion of the ears have been noted in large populations of Rye (Kostoff, 1940), Maize (Reeves and Stuvert, 1940), Sorghum (Karper, 1936; Ayyangar and Sankara Ayyar). In these plants also the separated plantlings produced new adult plants. This phenomenon has been adduced to be due to environmental influence in all the above cases and in some easily reversible. In the natural populations of ragi also these mutations were noted in one particular strain (E.C. 3517—3 plants in 200,000).

It is interesting that this same effect has been produced by X-rays and in one of the X_3 progenies of this lot the same kinds of plants were again met with (out of a total population of 103, 5 were with ears completely foliated and 6 with main ears alone abnormal, partially foliated and partially fertile), indicating that this could be heritable also (probably involving 3 factors giving a 63 : 1 ratio).

(*c*) *Truncated panicles* (Fig. 4):

In this type the peduncle becomes much broadened and the individual spikes are reduced in length. The spikes appear as though the top 1/3 had been cut off to a level. The number of spikelets is reduced and they are branched. Partial sterility occurs. This type has bred true.

II. Pennisetum typhoides Stapf. & Hubbard (*Cumbu*)

(*a*) *Weak midribbed plants* (Fig. 5):

Amongst the X_2 progeny one culture was found segregating for plants with well-developed midribs and plants with weak or no midrib. In normal plants the blade develops a midrib which keeps the leaf supported more or

less horizontally. When the midrib is not developed or is weak, on the other hand, the leaves droop and hang down. A slight amount of midrib tissue may be developed towards the base of the leaf blade. The midribless plants are completely sterile. The proportion of normal plants to the weak midribbed was 150 : 27. This ratio is much nearer to 13 : 3 (143·8 : 33·2) than to 3 : 1 (132·75 : 44·25). In the X_3 generation again the weak midrib plants were far in defect of the expected ratio. Cumbu being a protogynous plant, it is difficult to prevent contamination and this naturally much disturbs the ratio. In *S. sudanense* (*l.c.*) similar weak midribbed plants have been recorded, with a proportion of 3 normal to 1 weak midribbed plants. The inheritance of this character in cumbu is further being studied.

(*b*) *Male sterile* (Fig. 6):

Male sterility was induced in one progeny as a result of X-raying. The anthers were white in colour and produced very little pollen. They were shrivelled and did not become pendant as in the normal ears, so that they presented a characteristic appearance. The counts on X_2 and X_3 progenies gave 112 male fertile plants to 35 male sterile plants (expected on 3 : 1–110·25 and 36·75 respectively). The gene M_s is a simple dominant to m_s (male sterile).

(*c*) *Gappy panicles* (Fig. 7):

Normally the panicles bear the spikelets continuous from the base to the tip. The spikelets are arranged in close and consecutive whorls on the main axis. In the gappy panicles this consecutiveness is broken by zones completely bereft of all spikelets, and thus exposing the main axis here and there. Several grades of this type of sterility are shown in the figure. The gappy character (g_p) behaves as a simple recessive to the normal one (G_p) (159 normal panicles : 55 gappy ones. Ca. on 3 : 1 = 160·5 : 53·5). In *E. coracana* (Krishnaswamy, *l.c.*) a monogenic segregation with the normal condition dominant was obtained in one family. However, the progenies of several other crosses effected between Normal and Gappy panicled plants gave a series of intermediates and when quantitatively considered it was estimated that at least 3 factors were involved. The gappiness in ragi appeared at a definite place and lent inself to quantitative determination. In cumbu, on the other hand, there is no definiteness as to the place of incidence of the gappiness.

(*d*) *Forked panicles* (Fig. 8):

The panicle normally is cylindric and tapering only at the apex. The apex may be sometimes short and blunt or it may prolong much attenuated

bearing a few vertically disposed, adpressed spikelets. Sometimes it is found to branch and the branching or forking may extend downwards beyond the middle of the panicle. Such panicles often appear as twin panicles. Both of them are completely fertile and give the same degree of grain setting. Rarely it might fork once more giving three branches. This phenomenon is very commonly observed in normal populations occurring on stray plants. Further the forking may not appear in all the panicles in a plant. In one of the X_2 families, however, a greater proportion of the plants with forked panicles was observed. The incidence was from rudimentary to almost complete twinning. Counts taken in this progeny gave 75 with all panicles normal and 36 with forked panicles suggesting a single factor difference. This phenomenon is being further studied.

(*e*) *Goose-necking of peduncles* (Fig. 9):

Goose-necked peduncles have been recorded in *Sorghum* (Ayyangar and Ponnaiya, 1941), where they are of very common occurrence. In *Pennisetum* this feature is a very rare phenomenon. Segregations were observed in X_3 progenies for this character. The counts gave (total of two families) normal 36 : goose-necked 18, indicating a single factor difference while in another family 47 goose-necked to 33 normal panicles were counted. The behaviour requires further pursuit.

(*f*) *Tip sterility* (Fig. 10):

In one of the X_2 progenies plants were met with in which the panicles were short but stout and the central axis was seen protruding beyond the panicle. In normal panicles this is fertile and the axis is not seen naked beyond the apex of the panicles. Shorter lengths of sterile axis are found at the apex, probably facilitating the emergence of the panicles. In the present case, however, the sterile portion measured about 5·1 cm. The analysis of the population gave 54 normal panicles and 32 tip-sterile ones (ratio agrees with 9 : 7 than with 3 : 1), indicating interaction of complementary factors.

(*g*) *Chlorophyll deficienceis*—(*i*) *Albinism*:

Albino seedlings were met with in only a few of the X_2 and X_3 cultures. In a few families of X_3 yellow seedlings also occurred. The incidence of this character has already been reported in natural populations (Ayyangar and Harihara Ayyar, 1935; Ayyangar, 1934). Both single factor and duplicate factor segregations were met with. Apart from these albino seedlings sectorial chimera (Fig. 11) expressed as partially albinotic occurred. Cases of albino striping, partly green and partly white leaved, were also met with.

In one case a thin white line was noted just within the margin running the whole length of the leaf. Very rarely streaks of white appeared in the middle.

(ii) *Golden yellow plants.*—A few plants were met with which were golden yellow in colour. This colour was most prominent in the mechanical tissues especially round the veins so that the midrib, the internode, the peduncle, bristles and glumes showed the colour predominantly. Thirteen genotypically different types of golden plants have been reported in Maize (Eyster, *l.c.*). The plants are fertile. The behaviour of this character is being further studied.

(*h*) *Suppression of leaf-blade* (Fig. 12):

The normal cumbu plant has fairly large wavy leaves. Types were found in which the blade was reduced equally on either side of the midrib, while the midrib itself was normal. In one instance the blade was suppressed on only one side of the midrib while on the other side it was normal. On the suppressed side just a flange along the midrib was all that was left of the blade. It appeared as though the two blades had rolled themselves on each other.

In one plant the panicle was found completely modified into a tubular leafy growth (Fig. 13). In another plant at the base of the peduncles was a bunch of leafy growth arranged fan-like and from this arose as a branch a long peduncle at the end of which was a normal panicle (Fig. 14).

Apart from the few abnormalities noted above, mutations of economic value such as early maturity and differences in panicle shapes in ragi and length and thickness of panicles, size of grains and leaf size in cumbu were also noted.

Mutations affecting the chlorophyll characters are the most common and those of the panicles come next. Least affected are the growth forms and the vegetative characters. Male sterility occurred in a number of cases in the X_2 generations in ragi, while in cumbu it was rare.

Grain colour in ragi is determined by three factors (S, B_1 and B_2). SB_1B_2 is a purple plant with brown grain. A factor D deepens the effects of the brown factors (Ayyangar *et al.*, 1931). Under the present heavy dose of X-rays no mutations occurred for grain colour in any of the numerous progenies examined.

The panicle shapes in ragi, though also determined by three factors (Q, E_1 and E_2; Ayyangar *et al.*, 1932) easily mutated, giving incurved, fisty and open panicles, while the glume lengths (three factors—Gl_1, Gl_2 and

Gl_3; Ayyangar and Warier, 1936) gave in only a few cases longer glumes in the X_1, but reverted to the normal condition in the X_2 itself.

In *D. melanogaster* it has been found that " different genes have different mutation rates and consequently different degrees of stability as regards those factors which produce " spontaneous " mutations. The X-ray work with *drosophila*, *antirrhinum* and Maize shows that the frequency of induced changes is also different for different genes. Different allelomorphs of the same genes may also show different degrees of stability in X-ray experiments (Timoféeff-Ressovsky, 1934). Thus in ragi the brown factors and the glume-length factors appear to be more stable than either panicle shape or chlorophyll factors.

The number of recognisable mutants was greater in cumbu than in ragi. Cumbu threw out mutations in all directions. Ragi is a tetraploid species, while cumbu is diploid. Stadler (1929) from his experiments on *Avena*, and *Tricticum* obtained a high rate of mutations in the diploid species and to a lesser degree in the tetraploid, while he obtained no mutations at all in the hexaploid species. Timoféeff-Ressovsky (*l.c.*) concludes from Stadler's data " in polyploid species most of the genes are present in double (or triple) number so that most of the recessive mutations cannot manifest themselves even in homozygous conditions as they are covered by the normal allelomorphs present in the other homologous chromosomes.....the doubling of the set of chromosomes can mask the detection of mutations. " Further he mentions that mutations become difficult of observation due (i) " Karyotypic masking " due to doubling of chromosomes, (ii) " Genotypic masking " due to specific suppressors, and (iii) " Phenotypic masking ". Stadler (1936) gives " the characteristic effects which may be expected in polyploid species as (1) a reduction in the apparent frequency of gene mutations, due to the presence of duplicate genes, (2) a reduction in the frequency of induced partial sterility due to the survival of deficient gametophytes, (3) the appearance of variations due to the phenotypic effects of the deficiencies and duplications which have been transmitted because of polyploidy, (4) the appearance of apparent recessive mutations, due to the loss of segments bearing a dominant gene which is present in only one set of chromosomes ". It is possible that these causes have contributed to a fewer number of mutants being thrown out in ragi.

Summary

1. Some of the recessive abnormalities induced by X-rays in *E. coracana* Gaertn. and *Pennisetum typhoides* S. & H. are described.

2. In *E. coracana* are described a chlorophyll deficiency in which the first two or three leaves are green and white banded, but the mature plant is fully green and two mutations effecting the panicle. In *P. typhoides* have been noted gappiness, tip-sterility, forking and goose-necking in the panicle, male-sterility, and weak-midribbed leaves all behaving as recessives.

3. While some of the plant characters in both these millets mutated easily, others showed no tendency to mutate. Thus in *E. coracana* the panicle shapes and chlorophyll factors mutated while the grain colour, length of glume and growth factors did not give any mutations, while in *P. typhoides* the greatest number of mutations were observed in the chlorophyll and panicle characters.

4. *P. typhoides* threw out more mutations than *E. coracana* and this is adduced to the diploidy of the former and the tetraploidy of the latter.

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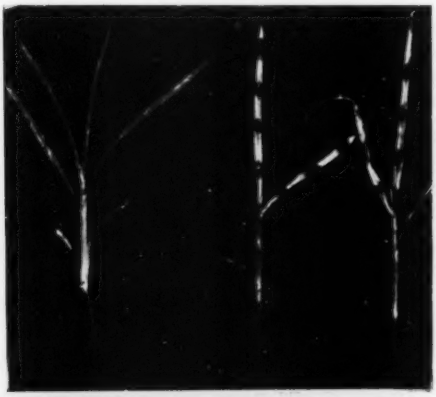


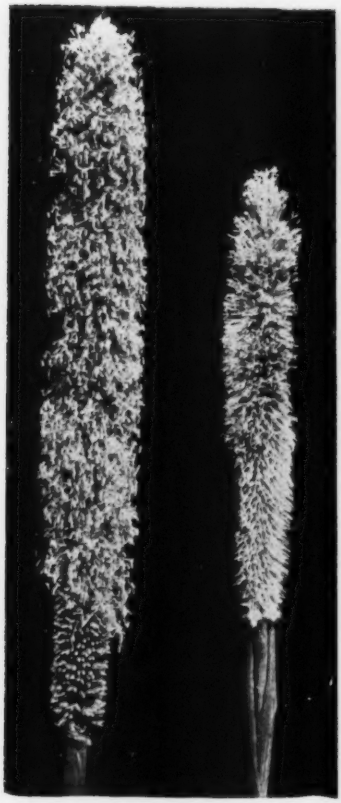
FIG. 1

FIG. 4

FIG. 2

FIG. 5

FIG. 3



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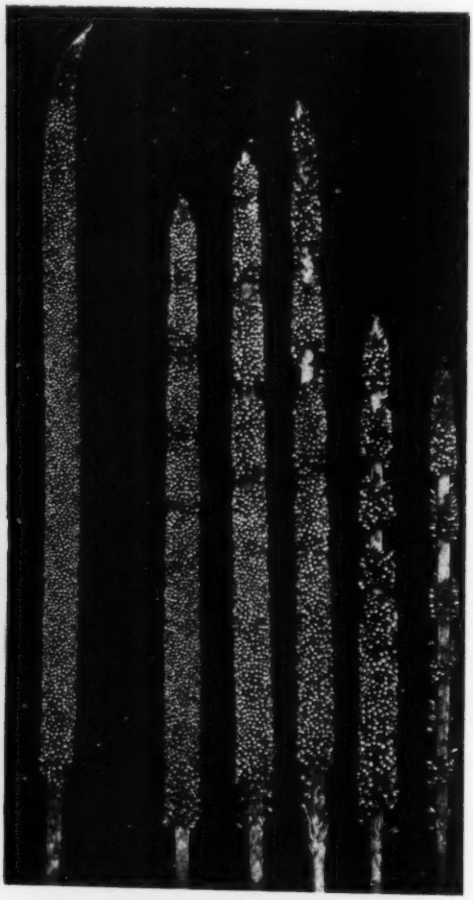

FIG. 7

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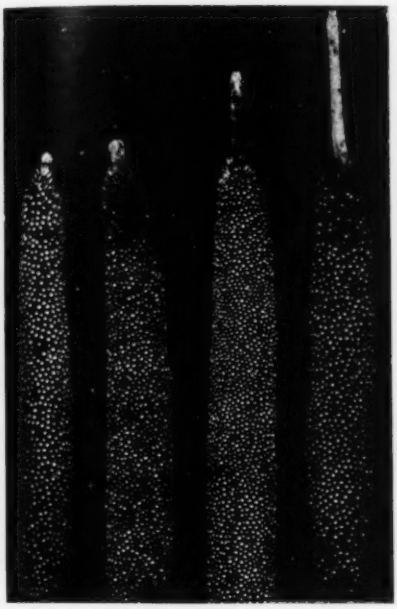
FIG. 9



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EXPLANATION OF FIGURES

FIGS. 1-4. Ragi

Fig. 1. Green and banded seedlings. Fig. 2. Upper half of a plant with foliated panicles. Fig. 3. One of the branches separated out showing the modified glumes and lemmas and the roots at the base. Fig. 4. Truncated panicle.

FIGS. 5–14. Cumbu

Fig. 5. Weak-midribbed plant. Fig. 6. Normal (left) and sterile anthers (right). Fig. 7. Normal ear (left) and types of gappiness. Fig. 8. Forked panicles. Fig. 9. Normal and goose-necked panicles. Fig. 10. Types of tip-sterility. Fig. 11. Green and white striped plants. Fig. 12. Plant with leaf-blade suppressed. Fig. 13. Plant with panicle suppressed. Fig. 14. Partially foliated and partially normal panicle.

THE DISCOGLOSSID SKULL

By L. S. Ramaswami

(Department of Zoology, University of Mysore, Intermediate College, Mysore)

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(Communicated by Dr. A. Subba Rau, D.Sc., F.A.Sc.)

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Introduction

The object of this short paper is to place on record the cranial morphology of two Discoglossid genera, *viz.*, *Bombina* Merr. and *Alytes* Wagl. and to find out if they show any resemblances to the Liopelmid examples.

Family Discoglossidæ comprises the three genera *Discoglossus* Otth., *Bombina* Merr. and *Alytes* Wagl. and under Liopelmidæ Noble (1931) included *Liopelma* Steindachn and *Ascaphus* Stejneger. The separation of *Liopelma* and *Ascaphus* from Discoglossidæ and the creation of a separate family Liopelmidæ for accommodating the two by Noble has been sufficiently established from cranial morphological grounds by Wagner (1934*a*) but de Villiers (1934, p. 35) remarked that *Ascaphus* " might reasonably be considered a Discoglossid ". Parker (1881) delineated the skulls of *Alytes* and *Bombina* while Boulenger (1897) figured *Discoglossus*. I have examined by sectioning the skulls of *Bombina igneus* Günth. and *Alytes obstetricans* Laur.

Observations

The naso-ethmoid region.—The anterior sectional views reveal the occurrence of cartilago prenasalis superior and inferior supporting the premaxilla and the profusely developed glandulæ intermaxillaris,—structures seen also in other anuran examples.

The Discoglossid examples lack both Gauppian Wulst. In *Ascaphus* (de Villiers, 1934) the larger Wulst is absent and the smaller is represented; in *Liopelma* (Wagner, 1934*a*) both are absent.

The plica obliqua depends into the cavum principale from cartilago obliqua in *Alytes* and *Bombina*. In *Ascaphus* and *Liopelma*, it depends from the tectum nasi.

The recessus sacciformis is uniformly developed in the Discoglossidæ. In *Alytes* and *Bombina*, it is noticed that posterior to the naris the plica obliqua separates the cavum principale from the lateral chamber (which is the anterior extension of recessus sacciformis). Both these, the cavum principale and recessus sacciformis gain entrance into the cavum medium (which is comparatively smaller than in other Anura investigated except the Pelobatid *Scaphiopus*, Ramaswami, 1935) and the lateral chamber is separated off as the nasolacrimal duct (arising from cavum medium) when the cavum principale opens into cavum inferius. This arrangement is schematically represented in figures 29 *a–c* of *Alytes* by Helling (1938, p. 632). According to him *Discoglossus* also exhibits the same arrangement. de Villiers (1934, p. 10) noted that neither the recessus sacciformis appertaining to the vestibule, " nor that appertaining to the cavum medium (Gaupp, 1904, fig. 140) is represented in *Ascaphus* ". *Liopelma* (Wagner, 1934 *a*) also lacks it.

A recessus olfactorius is described by Rowedder (1937) and Helling (1938) in the Discoglossidæ. On page 591, the latter author noted :

> Der Recessus olfactorius grenzt medialwärts an das Riechelepithel der Haupthöhle, lateral an die Plica terminalis bsw. an das Vorderende der seitlichen Nasenrinne.

Associated with the r. olfactorius, a set of glands,—glandula oralis interna is described. These glands open into the recessus. While the recessus and glands are feebly developed in *B. igneus*, fairly well in *Alytes*, they attain their maximum development in *Discoglossus* (Helling, 1938, fig. 6). De Villiers (1934) and Wagner (1934*a*) do not refer to a recessus or oral glands in *Ascaphus* and *Liopelma*. Obviously they are absent. If so, along with the Liopelmidæ, these two closely associated structures are also absent in Brachycephalidæ, Ranidæ, Polypedatidæ and *Acris* and *Pseudacris* (Hylidæ). In the other families investigated by Helling, *viz.*, Pelobatidæ, Bufonidæ, Hylidæ, Cystignathidæ and Microhylidæ (*Microhyla* ?) the recessus and g. oralis interna are noticed.

The glandulæ nasalis lateralis are ranid in disposition in the Discoglossidæ. Situated below the planum terminale cartilage, the duct of these

gains entry into cavum principale (Haupthöhle of Helling, 1938) internally to the septomaxilla in the plica below the cartilago obliqua. Only in *Bombina* the glands extend as far as the choana. These glands are absent in *Ascaphus* (de Villiers, 1934, p. 13) and are present in *Liopelma* (Wagner, 1934*a*) and are conspicuously absent in aglossal Anura (Helling, 1938).

The recessus medialis or Jacobson's organ of the cavum inferius is peculiarly disposed in *Bombina*. It is not differentiated into a highly nucleated lateral region towards septum nasi as in *Rana* but the entire portion lying below the cavum principale is the anterior portion of the organ. The cavum principale opens into this posteriorly. More posteriorly the epithelium of the recessus lateralis is distinguishable ; a large tonsillar patch is noticed in this. Obviously this is a primitive arrangement. In *Discoglossus* and *Alytes*, the organ is as in *Rana*. In *Ascaphus*, the r. medialis is large and in *Liopelma* the Jacobson's organ is found to be continuous with r. lateralis anteriorly and only in one section it is demarcated.

Opening into the Jacobson's organ are the glandulæ nasalis medialis or Jacobson's glands. In *Bombina* the g. n. medialis appear below the solum nasi and above the g. intermaxillaris. Posteriorly they pass through the basal fenestra and enter the region of Jacobson's organ. This is also noticed by Müller (1932) in *Acris*, *Hyla*, *Rhacophorus* and *Discoglossus* but he considered this as a part of intermaxillary gland when he said (p. 139) :

> Ausserdem konnte ich bei *Acris*, *Hyla arborea*, *Rhacophorus* und *Discoglossus* (die Tiere sind in progresiver Reihe geordnet) ein Eindringen der Intermaxillardrüse in den Recessus medialis der Nasenhöhle feststellen.

Müller obviously followed Wiedersheim (1876) who incorrectly described in *B. igneus* the lower nasal glands as a " darkly staining, upper part of glandulæ intermaxillaris ". However, the g. n. medialis can be easily identified from the lower g. intermaxillaris as Born (1876) recorded. I have also seen the occurrence of median glands below septum nasi and its penetration into the r. medialis region in *R. curtipes* (Ramaswami, 1935*a*). The medial nasal glands occur further posteriorly below the cavum principale also. In *Ascaphus* the medial nasal glands are particularly large and coiled and open into the posterior diverticulum of " r. medialis cavum inferius " (Jacobson's organ). In *Liopelma* the r. medialis is surrounded by the g. n. medialis on the septal side. The opening is not described. Whether these glands appear anteriorly and penetrate through the nasobasal fenestra as in *Bombina* and *Discoglossus*, it is not known.

The glandulæ intermaxillaris are abundantly developed in the Discoglossidæ. In *Alytes*, where there is a prechoanal sac some of the glands open into it ; the lateral portion situated below the plica (near the maxilla) extend as far as the choana. The lateral part of the intermaxillary gland is absent in *Discoglossus* (Müller, 1932). *Bombina* has no prechoanal sac, probably also *Discoglossus*.

In the palate of *Bombina*, a set of glands which Müller (1932) described as " mucous membranes grooves " composed of ciliated and goblet cells open into the mouth. Reichel (1881) compared them with lateral palatal glands of Reptiles. No reference is made to this by Helling (1938).

The Rachendrüse or palatal glands which appear in the Discoglossidæ (Müller, 1932) below the planum terminale (oral part of glands) and eminentia (median part) in the internal naris region, postchoanally unite to form a broad patch. The oral part of the glands open by a few ducts into the choanal region. In *Ascaphus* and *Liopelma* the oral and median parts of the gland are *separate* and the lateral one opens into the cavum principale dorsally to plica and not into choana. Wagner (1934 *a*) also noted the choanal opening of lateral glands in *Bombina*.

The Bursa angulris oris is found in *Discoglossus*, *Bombina* (Fuchs, 1931; Wagner, 1934*a*) and in *Alytes*. In the Liopelmid genera it is absent.

The septum nasi is short and not broad [though Parker (1881) described a broad solum in *Bombina*] in all the three genera. On account of the incomplete approximation of nasals, the septum is visible dorsally in *Alytes*, *Bombina* and *Discoglossus*. In *Ascaphus* (de Villiers, 1934, p. 8, fig. 2) a broad and short septum is depicted ; Wagner's figures give an impression that the septum is short in *Liopelma*.

The septomaxilla (membrane bone) is uniformly met with in *Alytes*, *Bombina* and *Discoglossus*. A limb of it enters the plica obliqua which depends into the cavum principale in *Alytes* and *Bombina* (Bruner, 1901). This is a feature also noticed in some bufoninæ (Ramaswami, 1937) and *Hyla* (Bruner, 1901). In the region of the origin of the nasolacrimal duct from cavum medium, the septomaxilla clasps the former in the Discoglossidæ. Parker (1881), however, denied the existence of this bone, obviously missing it in macerated skulls.

The sphenethmoid ossification starts in the posterior part of nasal septum [*presphenoid*, which Parker (1881) was not able to see] in the choanal region in *Bombina* and invades the sides and floor ; however, the trabecular floor is met with posteriorly and this cartilage bone is seen as investing internally and outside the trabecular trough and even this is lost

posteriorly when only the cartilaginous skeleton is seen. In *Alytes* the presphenoid ossification is met with ; the cartilaginous sides are invested both internally and outside with bone which persists posteriorly in a large number of sections. In *Ascaphus* (de Villiers, 1934) it is not known if the sphenethmoid starts as an ossification in the septum nasi which it does in *Liopelma* (Wagner, 1934*a*). In the latter, even the antorbital portion is feebly calcified.

A palatine (Parker, 1881; Boulenger, 1897) is wanting in both *Alytes* and *Bombina*.

The orbito-temporal region.—There is a large frontoparietal fontanel in *Alytes*, *Bombina* and *Discoglossus* (Parker, 1881; Boulenger, 1897). In *Alytes* (Parker, 1881) in the *optic* region of foramen opticus there is a tænia, a transverse cartilaginous commissure connecting the orbital cartilages; similarly in *Bombina*. Probably Parker missed this in *Bombina* for he does not refer to it.

The presence of a supraorbital cartilage in *Alytes* has been described by Parker (1881) and the development of the same has been worked out by Stadtmüller (1931). A similar structure is also noticed in *Phyllomedusa bicolor* (Parker, 1881).

The eminentia olfactoria is poorly represented in *Discoglossus*, *Bombina* and *Alytes*. However, the eminentia is more elevated in *Alytes* than in the other two congeners, and Noble (1931) also noted that *Alytes* was more terrestrial than the other examples. Helling (1938) brought the Discoglossidæ under his group II where the eminentia was not elevated. He came to the conclusion that in aquatic genera the eminentia was absent and only terrestrial forms developed it. This is not always the case for, I have shown that in *Rana hexadactyla* (Ramaswami, 1935) which is typically aquatic an eminenta is well developed. Exceptions are not, therefore, uncommon and I suppose that in a majority of terrestrial forms according to Helling (1938), it is present.

The nasolacrimal ducts open below the eye by two ductules as in ranid examples.

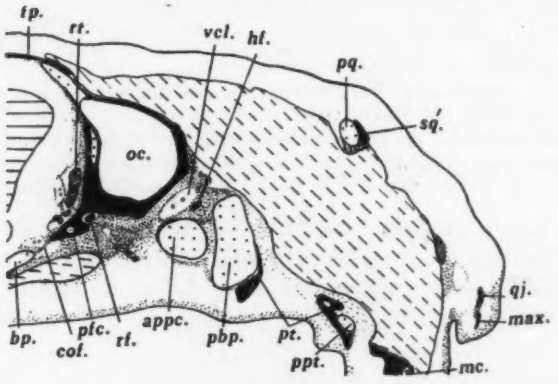
The fourth nerve gains exit in the orbital cartilage; the large optic foramen is seen in the side wall. The pila metoptica reduces itself to a round cartilage situated between the prootic foramen above and the metoptic (plus carotid) foramen below. In the prootic foramen the vena capitis medialis, the ramus ophthalmicus profundus and the abducens nerve and in the lower, the ophthalmica magna artery and the oculomotor nerve are

seen. Obviously the bridge separating the carotid and oculomotor foramina has broken down, a feature also seen in the bulk of frogs (Gaupp, 1906).

In *Alytes* also, the pila metoptica extends as far as the prootic foramen.

The occipito-auditory region.—In *Bombina*, it is noticed that the trigeminal ganglion appears first in the prootic foramen (text-fig. 1, *tg.*) and only posteriorly the geniculate ganglion is situated. Both ganglia are separate (*see* text-fig. 4). The origin of palatine nerve from geniculate ganglion is met with and in figs. 1 and 2 the anterior cartilaginous postpalatine commissure (*appc.*) with which the pseudobasal process (text-figs. 2, 3, 4, *pbp.*) articulates is cut ; thus there is no basitrabecular process.

TEXT-FIG. 1



TEXT-FIG. 2

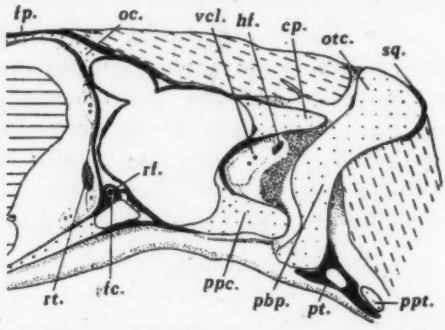
TEXT-FIG. 1. Transverse section of *Bombina igneus* Günth. in the region of the trigeminal foramen. × 30. *aci.*, arteria carotis interna ; *appc.*, anterior portion of postpalatine commissure ; *cpi.*, cartilaginous pila separating the prootic and metoptic foramina ; *fp.*, frontoparietal ; *tg.*, trigeminal ganglion.

TEXT-FIG. 2. Transverse section posterior to text-fig. 1, showing prefacial commissure. × 30. *appc.*, anterior portion of postpalatine commissure ; *bp.*, basal plate ; *cof.*, foramen for internal carotid artery and oculomotor nerve ; *fp.*, frontoparietal ; *hf.*, r. hyomandibularis facialis ; *max.*, maxilla ; *mc.*, Meckel's cartilage ; *oc.*, otic capsule ; *pbp.*, pseudobasal process ; *pfc.*, prefacial commissure ; *ppt.*, processus pterygoideus ; *pq.*, pterygoquadrate ; *pt.*, pterygoid ; *qj.*, quadratojugal ; *rf.*, root of facial ; *rt.*, root of trigeminal ; *sq'.*, zygomatic process of squamosal ; *vcl.*, vena capitis lateralis.

Further, the two ganglia (text-figs. 2, 4) are separated by a bony prefacial commissure (*pfc.*),—the cartilage (text-fig. 1, *cpi.*) which separated the prootic from the metoptic merging with the prefacial commissure (text-fig. 4, *apfc.*). The metoptic foramen (*cof.*) is still visible in fig. 2.

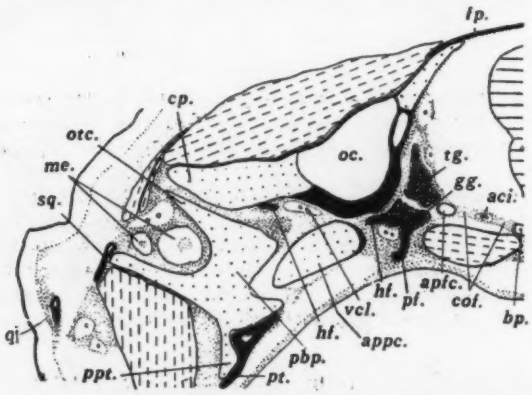
The otic capsule has grown so far forwards that the anterior wall of it (text-fig. 2, *oc.*) has merged with the prefacial commissure (*pfc.*). The

root of the facial nerve (*rf.*) is accommodated in a niche in the ventral portion of the otic capsule and posteriorly it enters the facial canal (text-fig. 3, *fc.*). The facial canal is situated in a bony bridge (text-fig. 3) running between



TEXT-FIG. 3. Transverse section posterior to text-fig. 2. The degenerate Eustachian tube, middle ear and otic connexion appear posteriorly. ×30. *cp.*, crista parotica; *fc.*, facial canal; *fp.*, frontoparietal; *hf.*, r. hyomandibularis facialis; *oc.*, tectal cartilage; *otc.*, processus oticus cartilage; *pbp.*, pseudobasal process; *ppc.*, postpalatine commissure; *ppt.*, processus pterygoideus; *pt.*, pterygoid; *rf.*, root of facial; *rt.*, root of trigeminal; *sq.*, squamosal; *vcl.*, vena capitis lateralis.

the otic capsule and the basal plate which is the postpalatine commissure. Further the anterior cartilaginous postpalatine projection seen in figs. 1, 2 and 4, arises from the base of the otic capsule (text-fig. 3, *ppc.*) in the postpalatine region as seen here and projects anteriorly as in *Liopelma*.



TEXT-FIG. 4. Transverse section of *Bombina ignea* Günth. in the middle ear region; right side of the animal, posterior to otic connexion. ×30. *aci.*, arteria carotis interna; *apfc.*, anterior portion of prefacial commissure; *appc.*, anterior portion of postpalatine commissure; *bp.*, basal plate; *cof.*, foramen for internal carotid artery and oculomotor nerve; *cp.*, crista parotica; *fp.*, frontoparietal; *gg.*, geniculate ganglion; *hf.*, r. hyomandibularis facialis; *me.*, middle ear; *oc.*, otic capsule; *otc.*, processus oticus cartilage; *pbp.*, pseudobasal process; *pf.*, r. palatinus facialis; *pt.*, pterygoid; *ppt.*, processus pterygoideus; *qj.*, quadratojugal; *sq.*, squamosal; *tg.*, trigeminal ganglion.

In *Alytes*, in the region of the foramen pro-oticum the trigeminal ganglion is noticed dorsally (with the oculomotor running internally and the abducens ventrally) and the facial ganglion appears posteriorly. Thus there is no connexion between the facial and trigeminal ganglia unlike what Pusey (1938, p. 537) depicts to be. There is a thin bony prefacial commissure. Sections show the hyomandibular nerve running anterodorsally to the postpalatine commissure. Like *Bombina** this also enters a canal (between the otic capsule, prefacial and postpalatine commissures) to reach the seat of origin.

During the development of the ear in *Alytes*, van Seters (1922) depicted that the otic capsule was incomplete towards the basal plate (p. 432, fig. 6) and later (figs. 7 *a–b*) the wall was formed and the root of the facial ran inside the otic capsule. In the 53 mm. stage of *Alytes* (p. 426, figs. 3 *a–h*) the root of the facial nerve ran in a canal and posteriorly along with the auditory as n. acustico-facialis.

De Beer (1937, p. 400) described that " In *Triton* (p. 182) and *Alytes* (p. 211) however, the medial wall of the auditory capsule in this region remains permanently or temporarily membranous, so that the cavity of the otic capsule appears to be bounded medially by the prefacial commissure and to be traversed by the facial nerve ". At any rate, in *Alytes*, the medial wall is formed [as van Seters (1922) also described] and the root of facial nerve runs in a facial canal formed by the prefacial, postpalatine and anterior basicapsular commissures.

With regard to *Ascaphus* and *Liopelma*, Pusey (1938) inferred that " like the Urodeles, their auditory capsules undoubtedly reach morphologically farther forward over the facial nerve and in addition they possess three foramina for the entrance of the VIII nerve into the capsule—another Urodele feature..... ". In Urodela, the head is composed of six segments barring the condyles (three metotic) while in Anura, there are only five (two metotic) segments. The reduction by one segment in Anura is correlated with the absence of " basitrabecular process, etc.," by Pusey (1938, p. 540). Further research can only settle whether the Liopelmidæ have included three metotic segments in their cranium or not ; however, in *Bombina* and *Alytes*, the otic capsule has projected anteriorly as in Urodela and has three acustic foramina but Götte (1875) described *two* metotic segments in *Bombina* (when interpreted in the light of modern knowledge). Van Seters (1922) accepted this when he said (p. 394) :

* A passing reference to the facial canal is made by Gaupp (1911, p. 422) in *Bombina* and *Alytes*.

> En me basant sur les résultats fournis par *Bombina*, *Bufo*, *Pelobates* et *Alytes*, je crois pouvoir sourtenir l'opinion, que chez les *Anoures deux somites postauditifs* seulement prennent part à la formation de la tête.

The middle ear region of *Bombina*, *Alytes*, *Discoglossus*, *Barbourula*, *Liopelma* and *Ascaphus* present an interesting study. While the middle ear, Eustachian tube, annulus tympanicus and plectrum are totally wanting in *Ascaphus* and *Liopelma* (de Villiers, 1934; Wagner, 1934*a*), they are well developed in *Alytes*, *Discoglossus* and *Barbourala* (?). In *Bombina* the tympanic membrane and annulus tympanicus are wanting while the Eustachian tube and plectrum are poorly represented. However, Stadtmüller (1931) depicted a degenerate middle ear ; in my preparation on one side (text-fig. 4, *me.*) I have been able to see two independent cavities in *Bombina* representing the reminiscent middle ear. Numerous variations are described in connexion with the attachment and disposition of plectrum in *Bombina* [Stadtmüller (1931*b*), Iwanzoff (1894), Kothe (1910) and Litzelman (1923)] referred either to the disposition or the development of the operculum and plectrum. According to Iwanzoff, in *Bombina* the middle ear is absent, the Eustachian tube is better developed than in *Pelobates* and the anterior cornu of the hyoid attaches itself on the operculum. Kothe did not agree with Iwanzoff's observation of the ceratohyal attachment. Litzelman in studying the metamorphosis of *Bombina* noted that the anterior cornu of the hyoid came in contact and fused with the plectrum and that Iwanzoff had mistaken the operculum for the upper end of the hyoid.

The variations noticed in *Bombina* has been summarised by Stadtmüller (1931*b*) and they are :

1. the columella may be completely supressed when the anterior cornu of the hyoid has no dorsal attachment,
2. the columella may fuse with the anterior cornu of the hyoid and may not ossify (Litzelman, 1923),
3. the columella may be present as a small bony nodule,
4. the columella may fuse with the crista preopercularis as in Urodela and may ossify.

My preparations of *Bombina igneus* confirms the observations of Litzelman (1923), *viz.*, the fusion of the anterior cornu of the hyoid with the plectrum which is cartilaginous and disappears anteriorly to the operculum. The operculum is fused by its dorsal edge with the rim of fenestra ovalis.

This is in conformity with the observations of Stadtmüller whereas Litzelman noted that the entire operculum was fused.

In *Alytes*, as already remarked, the middle ear, tympanic membrane, annulus, Eustachian opening and plectrum are well developed. The pars externa plectri gives rise to a pars ascendens cartilage which attaches itself by connective tissue (or fuses) with the internal part of crista parotica. The occurrence of this commissural cartilage is noticed in some bufonin* examples (Ramaswami, 1937) and corresponds to the pars dorsalis of *Lacerta*. Kruijtzer (1931) delineated a process (p. 88, fig. 10) arising from the pseudobasal process (his processus basalis palatoquadrati) internally to the pterygoquadrate which probably is the otic process.

Further in *Alytes*, the plectrum is composed of three parts, partes interna, media and externa. But de Beer (1937, p. 212) denied the division when he said, " At all events, it is important to note that in *Alytes* there is no subdivision between internal and external parts of the plectrum, and that the dorsal end of the ceratohyal, after becoming detached from the hinder surface of the quadrate, becomes attached to the plectrum ".

In the occipital region Stadtmüller (1931) described the ossification in *Bombina pachypus* as *pleuroccipitale* and in *igneus* this bone merges with the prootic and the composite one is called *pteroccipitale*. A similar co-ossification is described by Boas (1914) in *Ichthyophis* (Apoda) where however, in addition to the occipitals, pleurosphenoid and parasphenoid also merge.

The roof (tectum) and floor (parachordal) of the cranium of both *Bombina* and *Alytes* in the otic region are cartilaginous.

The upper jaw.—The disposition of the pterygoquadrate and the bones connected with the upper jaw are also depicted in figs. 2, 3 and 4 ; the zygomatic process of the squamosal (text-fig. 2, *sq'*.) and lower down the quadratojugal (*qj*.) and maxilla (*max*.) are seen in fig. 2. In fig. 3 the hyomandibular nerve (*hn*.) running over the postplatine commissure (*ppc*.) and in fig. 4 which is posterior to the Eustachian passage, the pterygoid investing the processus pterygoideus (*ppt*.) and the ventral portion of the pseudobasal process (*pbp*.) are seen. Posteriorly the quadratojugal invades the quadrate cartilage partially.

The occurrence of " cartilago pararticularis " in *Bombina* was dealt with in detail by Stadtmüller (1931*a*). It is considered to be a cartilage of independent formation ventrally to the plectrum (p. 797, fig. 6) in the ligamentum suspensorio-hyoideum connecting the posterior end of the

* *Bufo vulgaris* (Ecke, 1934) lacks it.

quadrate and the dorsal portion (processus ascendens of hyale) of the ceratohyal.

The lower jaw.—In *Bombina* and *Alytes* the mento-Meckelian ossification is continuous with the dentary. Only in *Bombina* there is an internal ossification in Meckel's cartilage in the suspensorial region which probably represents an articular. I have not been able to see a similar ossification in *Alytes.*

In the hyoid of *Bombina* [*pachypus* (Fuchs, 1929) ; *maximus* (Blume, 1932) *igneus* excluded], *Discoglossus pictus*, *Alytes obstetricans* (Ridewood, 1898), *Liopelma* (Trewavas, 1933), *Ascaphus* (de Villiers, 1934), *Pelobates* (Ridewood, 1897 ; Fuchs, 1929), *Rhinophrynus* (Stadtmüller, 1936*a*) a parahyoid bone is described.

Summary

1. The Gauppian Wulst are wanting in *Alytes* and *Bombina*; they are also absent in Liopelmidæ.

2. A limb of the septomaxilla enters the plica obliqua in *Alytes* and *Bombina* and possibly in *Discoglossus.* This is not so in Liopelmidæ.

3. A recessus olfactorius associated with glandulæ oralis interna in the cavum principale is noticed in *Alytes* and *Bombina* ; probably they are absent in Liopelmidæ.

4. The g. nasalis lateralis and medialis occur uniformly in Discoglossidæ. In *Bombina* (*pachypus* and *igneus*) the medial nasal glands appear above the g. intermaxillaris and pass through fenestra nasobasalis ; the g. n. lateralis are absent in *Ascaphus.*

5. A prechoanal sac is present only in *Alytes* ; the Liopelmidæ lack it.

6. The Jacobson's organ (recessus medialis of cavum inferius) is not a *lateral* nucleated extension of c. inferius in *Bombina* as in *Alytes* and *Discoglossus. Ascaphus* and *Loipelma* resemble the ranid examples.

7. The palatal glands (Rachendrüse) encircle the choana posteriorly in *Alytes, Bombina* and *Discoglossus.* In Liopelmidæ the oral and lateral parts are separate.

8. The Discoglossidæ possess Bursa angularis oris ; the Liopelmidæ lack it completely.

9. The sphenethmoid arises as a presphenoid ossification in the nasal septum ; posteriorly it is paired. The paired nature is also seen in Liopelmidæ.

10. Both in *Bombina* and *Alytes,* the V and VII nerves are separated by a prefacial commissure ; the root of the VII passes through a facial

canal formed by the prefacial and postpalatine commissures and otic capsule. The postpalatine commissure extends anteriorly from the lateral side of the otic capsule which itself has projected far anteriorly. *Ascaphus* (de Villers, 1934) has a basitrabecular process and the root of the VII appears to traverse the otic capsule since the median wall of the otic capsule is not formed. In *Liopelma* (Wagner, 1934*a*) the hyomandibular nerve passes through the postpalatine commissure and the palatine nerve runs anteriorly. A prefacial commissure is absent.

11. The pterygoquadrate articulates by means of the pseudobasal process with the postpalatine process in *Alytes* and *Bombina*.

12. There are three acustic foramina in *Bombina* as in the Liopelmidæ.

13. In the specimen of *Bombina* investigated, there is a cartilaginous columella attached to the anterior cornu of the hyoid confirming Litzelman's observations. The middle ear is represented as two independent cavities on one side and on the other, there is only one. In *Alytes*, the three parts of the plectrum and a pars acsendens plectri are noticed. The Liopelmidæ lack the middle ear and plectrum.

14. An articular ossification in Meckel's cartilage of *Bombina* is noticed ; this is absent in *Alytes*.

Conclusion

It is clear that the Discoglossid skull exhibits features in which it differs from the Liopelmid. The possession of (1) a limb of the septomaxilla in the plica, (2) a recessus olfactorius, (3) a postchoanally united palatal glands, (4) a Bursa angularis oris, (5) a prefacial commissure, (6) a facial canal in the Discoglossidæ and their absence in Liopelmidæ is sufficient to treat them as independent families. The absence of middle ear, etc., in Liopelmidæ (from the view-point of auditory skeleton, de Villiers, however, considers the Liopelmidæ as not primitive) and *Bombina* can only be explained as due to parallelism since the absence of those structures also occurs in an unrelated genus *Hemisus*.

I wish to express my thanks to Prof. A. Subba Rau for helpful criticisms.

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IMPROVEMENT OF BRINJALS (*SOLANUM MELONGENA*, L.) BY SELECTION IN THE BOMBAY PROVINCE

By G. S. Cheema, D.Sc., I.A.S.,
Horticulturist to Government, B.P., Poona,
B. Nazareth, B.Ag.,
Agricultural Officer, I/C, Modibag, Poona,
AND
S. R. Dhareshwar, B.Ag.,
Agricultural Officer, Ganeshkhind Fruit Experiment Station, Kirkee

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1. *Introduction*

THE question of improvement of indigenous vegetables in India has assumed great importance during recent years. The quality of indigenous vegetables available in the Indian market is very poor. The cultivation also is not very paying. It is observed that the acreage under vegetable cultivation in several districts in this Province has fallen during recent years. The Department of Agriculture in the Province of Bombay realising the importance of this work, prepared a comprehensive scheme relating to the improvement of Brinjals and Chillies in the year 1930. This scheme was accepted and financed from the Sir Sassoon David Trust Fund. This paper deals exclusively with the improvement of Brinjals only by selection of strains which are superior both in quality and yield.

2. *Previous Literature*

Very little work has so far been done in India on the improvement in the quality and yield of indigenous vegetables. The Market Committee appointed by the Government of Bombay,[1] had laid great stress for such improvement. In foreign countries research on some of the important vegetables like chillies and brinjals has attracted some investigators. Most of such work, however, relates to systematic study of varieties, hybridization, inheritance of characters and the analysis of constituent parts. In India the improvement of brinjal crop by selection has not been so far undertaken anywhere. Balaji Rao[12] made an attempt to hybridize the common egg plant with a brinjal variety from Mysore differing greatly in general appearance. This work was done only for one generation.

A very interesting work relating to the improvement of egg plant by selection and hybridization has been carried out in the Philippines. The study of variations and selection of some varieties of egg plant is reported by Macabasco[8]; while Bayla[2] carried out hybridization of egg plant. Studies relating to floral biology and morphology of one of the types of egg plant have also been reported by Magtang.[9] In Puerto Rico, the inheritance of colour in egg plant has been studied by Nolla.[11] Halstead[5] also has studied colour variations in egg plant. Boswell[3] records observations relating to the improvement and genetics of egg plant along with other vegetables in the United States of America. In Japan, also, systematic breeding work has been carried out by some workers.[6,7,13] with good results. Seed catalogues of various countries show that some good quality of brinjal varieties are under cultivation there.

3. *Improvement of Brinjals* (*Solanum melongena*, L.)

During the year 1937–38, the total area under brinjal in the Bombay Province was 17,073 acres, the distribution in each division being as follows :—

	Acres	Percentage of the total acreage
Deccan	7,786	45·6
Karnatak	4,155	24·3
Gujerat	3,590	21·0
Konkan	1,542	9·1
TOTAL ..	17,073	100·0

(Figures for each district are given in Appendix I)

The district of Dharwar has the largest acreage, *i.e.*, 1,775 acres or 10 per cent. of the total. Poona has also nearly as much, *i.e.*, 1,758 acres. The other centres of importance are Satara (1,499 acres), Belgaum (1,331 acres), Sholapur (1,224 acres) and Surat (1,145 acres).

In Gujerat, varieties like ravaiya, round purple and the long purple are generally very popular. In the Karnatak, the Krishna Valley type is much appreciated. In the Deccan, however, the Dorli and the Gote are preferred to other varieties.

The work of Vegetable Research was entrusted to the Horticulturist to Government, B.P., Poona, and was located at the Ganeshkhind Fruit Experiment Station, Kirkee. One Agricultural Overseer* and Sub-overseer were appointed and the work was started early in 1931.

During the first four seasons, unit selection was done and high yielding biotypes were isolated. These were tested finally for yield during the next four seasons in order to get conclusive results before the best ones could be tried on field scale and subsequently distributed to the cultivators. The selected seed is now available for trial on the cultivators' field. Further work on this crop is, however, stopped.

A. Material

A collection of 45 representative samples from all the important brinjal growing centres in the Province was made and their history recorded

* Mr. S. M. Patel .. 13-1-1931 to 21-5-1932,
Mr. I. A. Sayed .. 21-5-1932 to 5-9-1932,
Mr. N. G. Masur .. 5-9-1932 to 31-3-1936,
Mr. S. K. Patwardhan .. 1-4-1936 to 30-9-1938.

(Appendix II). This collection includes three foreign types. With two more exotic types added, work was started with 47 samples. These samples were grown in duplicate plots in the rabi season of 1931.

B. Varieties of the Bombay Province

A systematic study of the different varieties of brinjal has not been made by any one in India. According to Gammie,[4] the majority of brinjals belong to the variety *esculenta* with all parts having prickles. The varieties mentioned by him are :—

(1) Purple, club-shaped fruits, a foot long. Common variety.

(2) Purple, large and almost globular fruits, diameter 8 inches.

(3) and (4) Purple or rarely white-small ovoid sorts.

Mollison[10] has grouped the Bombay brinjals into the three following classes according to their size and shape :—

(1) Purple, large fruits, tapering gradually from the attachment to flower stalk to a round, full thick end, 10″ to 12″ long. Common variety.

(2) Purple, large and round fruits, pomelo size.

(3) Purple, small nearly round or slightly oval. Orange size variety. Favourite of the Surat District.

The author further adds that the colour of fruit, classified as above, varies from purple to variegated purple and green or yellowish green, faintly marked with purple.

The study of the plant and fruit characters of the samples grown showed 35 distinct types. Half of these were smooth and the rest thorny all over. In regard to the latter character, Gammie[4] says, " the absence of defensive armour in the superior varieties may be the result of long continued cultivation ". In his opinion, European seeds usually give plants without prickles. Quoting Duthie, Mollison[10] says, " brinjal is often met with as an escape from cultivation in which condition it becomes prickly and more prolific as to the number of fruits". In our collection, the foreign varieties, the Gujerat types and almost all Konkan types are found to be smooth. The Deccan types, however, which mostly comprise the Dorli and the Gote are thorny and have remained so in spite of " long continued cultivation ".

An attempt has been made to classify the representative types in our collection. After a detailed study of the fruit characters they have been classed under 11 groups with several horticultural forms under each. The description of these varieties is given in Table I.

TABLE I

Classification of Brinjal Varieties

Group	Colour		Shape	Size	Group named as	Culture Nos. under these groups	Remarks
	Ground	Mottling					
I	Purple	..	Conical	Big	Foreign	3, 4, 5, 6, 7	Comprises the foreign types
II	do.	..	Medium to long	..	Long purple	13, 14, 21, 25, 35, 38, 39, 41, 42, 46, 48, 49	Long purple (Appendix II)
III	do.	..	Round or oval	Medium or small	Round purple	10, 15, 16, 36, 45	The " Surti " Round
IV	Reddish purple	White	Round	Medium	Round thorny	28, 31, 32, 34, 50	The " Gote " vangi of Deccan
V	Green	Purple patches	Oval or round	do.	do.	32–10, 34–27	Fruits slightly ridged
VI	do.	White	do.	Small	Small thorny	8, 20, 22, 26	The " Dorli " vangi of Deccan
VII	do.	Purple	Oval-round or elongated	do.	Green purple	27	do.
VIII	do.	White	Round	Medium	Round green	23	Fruits bigger than in group VI
IX	do.	White and purple	do.	do.	do.	11, 11 CH, 37	The " Krishna Valley " type
X	do.	Purple	Elongated	Fairly big	Long green	17, 18, 24, 40, 43, 44	Long green (Appendix II)
XI	Purple	Green or green and white	Round or elongated	Very small	Purple green	33	Dorli type but fruits very small

C. Method of Cultivation

Work on brinjal improvement was started in the rabi season of 1931. The usual tillage operations were given and the plot was manured with Farmyard Manure at the rate of 30 cartloads per acre. The seeds were sown on raised beds in the second week of September. The attack of aphis was controlled by spraying the seedlings with 2 ozs. of Fish Oil Rosin Soap in one gallon of water. The curly leaf and malformation of leaves which often occur in the early stages, were controlled by Black Leaf 40 (3/4 oz. Black Leaf 40, 5 gallons water and 3 ozs. bar soap).

The plants were irrigated at intervals of 8 days. The soil round-about the plants was stirred at different intervals and heaped round the plants. After about a month, it became necessary to control the curly leaf attack by spraying the plants with Black Leaf 40. The aphis attack at subsequent stages of growth was checked by spraying the plants with Fish Oil Rosin Soap.

D. Study of Plant and Fruit Characters

Plant growth records were maintained and promising plants were bagged. For the purpose of selection, the following ideals were kept in view :—

(1) High yield.
(2) Fruits with least number of seeds.
(3) Fruits having pleasant taste.
(4) Resistant to disease types.
(5) Brinjals having short maturity period.

Special attention was paid to the selection of round or oval shaped fruit of medium size with attractive colour.

The study of plant and fruit characters had been closely followed and selections exhibiting pure characters were bagged. In the year 1933–34, a detailed study of the following plant and fruit characters was undertaken.

(i) *Plant characters*
(*a*) Habit of growth
(*b*) Leaf size, shape and margin
(*c*) Prickles on plant parts
(*d*) Colour on plant parts

(ii) *Fruit characters*

(1) Yield per plant

(*a*) Length, diameter and weight

(*b*) Shape

(2) Fruit colour

i. Plant Characters

(*a*) *Habit of growth.*—A considerable variation in this character was noticed and the cultures under study were grouped into the following four grades :—

TABLE II

Habit	Culture Nos.	Type	Group Nos.
1. Tall	11–2–11	Krishna Valley	IX
	11 CH–9–4	do.	IX
	23–3–24	Dorli Vangi	V
	27–32–6	do.	V
2. Spreading	8–3–24	Dorli Vangi	V
	8 C–2–24	do.	V
	10–3–20	Surti Round	III
	15–31–20	do.	III
	28–21–24	Gote Vangi	IV
	32–7–3	do.	IV
	34–27–21	do.	IV
	32–10–3	do.	IV
3. Erect	25–32–22	Long Purple	II
	38–33–20	do.	II
	39–23–6	do.	II
	40–16–6	Long Green	X
4. Dwarf	3–3–22	Black Beauty	I
	7–1–5	New York Improved Purple	II

The plants grouped under the second grade do not grow tall but have a tendency to spread out. The plants of Dorli vangi falling under the first grade are slightly bigger in size than those under the second grade.

(*b*) *and* (*c*). *Leaf and prickles on plant parts.*—The different types of brinjals have their own characteristic leaves. The leaves vary in surface area and have either a wavy or nearly entire margin. In some types, the margin is lobed. There are two classes of brinjals so far as the character of prickles borne on plant parts is concerned. In one, the internodes, petioles and leaves are all prickly and in the other, they are totally absent.

The cultures belonging to these groups are shown below :—

TABLE III

Prickles on plant parts

Prickly	Type	Without prickles	Type
28–21	Gote Vangi	23–3–24	Dorli Vangi
32–7	do.	25–32–22	Long Purple
32–10	do.	38–33–20	do.
34–27	do.	39–23–6	do.
8 C–2–24	Dorli Vangi	10–3–20	Round Purple (Surti)
8–3–24	do.	15–31–20	do.
27–32–6	do.	3–3–22	Black Beauty
		7–1–5	New York Improved Purple
		11–2–11	Krishna Valley
		11 CH–9–4	do.
		40–16–6	Long Green

Gammie[4] describes the prickly type as *esculenta* and alludes to the presence of " prickles " on the under-surface of the leaves only. There are, however, some types in our collection having prickles on both the surfaces of the leaves. These are found in the following Gote and Dorli selections :—

Gote—28-21-24 ; 32-7-3 ; 32-10-3 and 34-27-21.

Dorli—8 C-2-24 ; 8-3-24 ; and 27-32-6.

(*d*) *Colour on the plant parts.*—There is a clear distinction between varieties in this respect. The parts mostly concerned are internodes, tender leaves, veins and prickles when present.

Internodes.—These are either green or purple with varying intensities of the latter.

Leaves.—These are generally tinged purple in the young stage, being deeper in types having purple internodes. As the leaf gets older, the colour changes to green or dark green so that there is little difference between adult leaves of different types. In the extreme, however, the adult leaves are very deep or dark purple with similar veins showing velvety appearance.

Veins.—The colour of the veins is either green or purple in varying intensities of the latter. Generally speaking, the colour is more intense on the vein in types having purple internodes.

Prickles.—These are similar in colour to the veins, being more intense. It may be stated in general that in the case of the coloured types (purple), there appears to be a regular gradation in the intensity of the purple colour from internodes to petioles, petioles to leaves, leaves to veins and

finally from veins to prickles, the last being the most intense. The strains representing this gradation are given in Table IV.

TABLE IV

Plant parts	Colour	Groups		
		I Foreign	II Long Purple	III Round Purple
1. Internode to petiole ..	Slightly Purple	3-3-22 7-1-5	25-32-22 25-32-27 38-33-20 39-23-6	10-3-20 15-31-20
2. Petiole to leaves	Purple	3-3-22 7-1-5	25-32-22 25-32-27 38-33-20 39-23-6	10-3-20 15-31-20
3. Leaves to veins	Deep Purple	3-3-22 7-1-5	25-32-22 25-32-27 38-33-20 39-23-6	10-3-20 15-31-20
4. Veins to prickles	Deep Purple	3-3-22 7-1-5	25-32-22 25-32-27 38-33-20 39-23-6	10-3-20 15-31-20

The remaining groups (Nos. IV and V—Gote Vangi ; VI, VII, VIII—Dorli Vangi ; IX—Krishna Valley ; X—Long Green and XI—Small Dorli) are green and have no colour on their plant parts.

Purple varieties are popular in Gujerat and non-purple ones in the Deccan and the Karnatak.

ii. Fruit Characters

(1) *Yield per plant*

(*a*) *Length, diameter and weight.*—In expressing this character, two factors, *viz.*, the total number of fruits and the average weight of fruits per unit area are generally taken into consideration. The latter varies according to the size of fruits. The character is given major importance in the selection of the best type.

In brinjals, the yield factor may be said to be a definite varietal charracter. The statement of yield, length of fruit and diameter of the different varieties is given in Table V.

TABLE V

Name of the variety	Length (cms.) Mean	Diameter (cms.) Mean	No. of fruits per plant	Weight of fruits (ozs.) Mean
Long Purple	24·16	7·15	12·7	8·66
Long Green	15·21	8·18	16·3	9·35
Round Purple	13·04	11·35	16·9	10·60
Round Green	10·79	10·31	12·5	8·76
Gote	10·38	7·50	24·6	4·93
Dorli	6·84	6·45	33·9	2·12

From the above table, it can be seen that the size (length and diameter) of an individual fruit has a great bearing on the average number and weight of fruits. These characters are considered as definite varietal characters. A significant correlation exists between the size and the average number of fruits per plant. The variety bearing smallest fruits, namely, Dorli, has the largest number of fruits.

(*b*) *Shape.*—There are almost innumerable forms so far as the shape of the fruit is concerned. Broadly speaking, the fruits may be grouped into the following classes so far as shape is concerned.

Conical.—This shape is met with in the foreign types. In these, the end of the fruit is almost flat with a slight depression so that the fruit can stand erect with its stalk upwards.

Long or elongated.—The fruit in these types is either straight or slightly curved at the stigma end and the length is more than twice the diameter.

Round.—The fruit appears round but the length is slightly more than the diameter and sometimes is nearly twice. In some cases, however, the fruits tend to bulge out in the centre and in such cases the length is less than the maximum diameter.

Oval.—In these types, the fruit appears almost egg-shaped and the length varies from 1·3 to 1·9 times the diameter.

(2) *Fruit colour*

The large number of horticultural forms met with in brinjal present equally variable shades of colour also. The more important ones are the following :—

(*a*) Purple in varying intensities.
(*b*) Green either light or deep.
(*c*) White.

The cultures, the fruit of which show colour variations mentioned above, are grouped in Table VI.

TABLE VI

Purple	Purple shining	Dark Purple	Dusty Purple
..	5–31–18 ; 6–2–25 ; 24–8–8	3–3–22 ; 10–3–20 ; 7–1–5 ; 15–31–20 ; 25–32–22 ; 25–32–27 ; 38–33–20 ; 39–23–6 ; 4–31–3	39–23–6

Green with White	Purple Green White	Green Purple	Pale Green	Green Purple White
8 C–1–2–10 8 C–1–1–3–7 8 C–2–24 23–3–24 11–4–28 23–10–25 23–34–24 11–2–11–22–3 11–2–11–22–8 19–33–7 23–10–2 20–7–12	8–3–24 30–18–23 27–32–6 27–32–6–10 26–5–32	40–16–6 40–16–14	23–14 11–8	11 CH–9–4–4 11 CH–9–4–13

Pink White	Reddish Purple White
23–10–25–4 8 C–1–1–2–7	28–8–21–21 ; 28–12–9–6 ; 28–21–24 ; 28–21–24–10 ; 32–10–3–3 ; 32–10–15 ; 34–27–21 ; 34–27–21–3

The above noted colours are present, very often, in different combinations and intensities. With experience, it is easy to distinguish between the ground or the main colours and the mottling or other shades. The mottling appears either as spots or in patches.

Very often, the spots are close to each other and appear as if they were in continuous lines. When mottling presents more than one colour, it is also possible to determine the intensity or prominence of one of them. Thus, green with purple and white means that purple occupies a larger proportion of the mottling. If the fruit is green with white and purple the colour white is more pronounced. These characters have been used in the classification of the eleven groups shown above.

4. Breeding by Selection

A. Line Culture Study and Selections

It has already been mentioned that all brinjal types, as a result of detailed study of various plant and fruit characters, are grouped in 11

classes. One type out of each group was selected for further studies. In addition to these, five more selections were included for further studies. So in all, the following selections were on hand for further work:—

Culture Nos.	Locality	Representing
	1. *Round type*	
10	Surti Round	Gujerat
11	Krishna Valley	Karnatak
17	Thana Local	Konkan
28	Gote Vangi of Poona	Deccan
32	do.	do.
34	Malvanki from Belgaum	Karnatak
	2. *Long type*	
25	Nasik Bengali	Deccan
38	Thana Pandhari vel Vangi	Konkan
39	Thana Kali vel Vangi	do.
40	Thana Pandhari vel Vangi	do.
	3. *Dorli*	
8 C	Poona Dorli	Deccan
8-3	do.	do.
23	Nasik	do.
27	Bengali	do.
	4. *Exotic variety*	
3-3	Black Beauty	Foreign
	5. *Exotic variety*	
7-1	New York Improved Purple	Foreign

These selections were thoroughly studied for their purity. On the basis of attractive colour, less number of seeds, medium size of fruit and yield, the following cultures were further selected for trials during the year 1932–33.

(1) 38 cultures representing the Deccan and the Gujerat tracts,

(2) 13 cultures representing the Karnatak tract,

(3) 23 cultures representing the Konkan tract, and

(4) 8 foreign types.

B. Detailed Study of Selections

After a detailed study, only twenty promising cultures out of the above collections were selected and their performance was recorded. The behaviour of these selections for the two years is given in Table VII.

TABLE VII

Statement showing the performance of some promising Brinjal types during 1932–33 and 1933–34

TABLE VII

Statemement showing the performance of some promising Brinjal types during 1932–33 and 1933–34

Culture Nos.	Tract	Average length of fruits (cm.)		Average No. of fruits per plant		Average yield per plant			
		1932–33	1933–34	1932–33	1933–34	1932–33 lb.	1932–33 oz.	1933–34 lb.	1933–34 oz.
				Group II—Long Purple					
25–32	Deccan	21·7	22·2	5·6	7·1	2	8	2	11
38–2	Konkan	21·3	25·2	12·7	13·2	5	11	3	3
39–23	do.	20·6	24·5	12·7	17·8	4	12	5	5
Local	do.	..	..	..	..	3	15	5	2
				Group III—Round Purple					
10–3–20	Gujerat	10·2	10·6	10·5	14·6	4	11	5	7
15–31–20	do.	10·5	11·2	4·8	20·1	2	12	8	4
Local	do.	..	..	..	..	4	7	5	12
				Groups IV and V—Gote type					
28–8	Deccan	9·6	8·1	12·6	35·7	4	0	5	8
28–21	do.	8·3	7·5	17·0	18·3	5	5	3	5
32–7	do.	8·9	8·4	19·2	28·6	5	12	7	3
32–10	do.	8·8	8·4	18·7	19·0	6	1	4	0
34–27	Karnatak	8·2	9·1	14·1	22·0	6	7	7	4
Local	do.	..	..	..	..	4	8	5	2
				Groups VI, VII, VIII and XI					
8 C–1–1	Deccan	8·8	7·2	22·4	39·8	2	2	3	6
8 C–2–24	do.	6·8	6·1	26·1	40·0	2	9	2	3
8–3–24	do.	3·9	4·6	37·7	47·0	1	14	2	4
23–3	do.	7·9	7·7	16·0	34·7	1	13	2	15
27–32	do.	6·4	6·3	27·5	21·2	4	0	2	6
30–18	do.	5·2	5·7	29·4	20·7	2	14	2	13
Local	do.	..	..	..	..	3	1	3	1
				Group IX—Krishna Valley type					
11 CH–4	Karnatak	7·9	8·3	12·2	10·2	4	4	3	2
11–2–11	do.	7·8	7·9	9·1	13·1	3	12	3	9
Local	do.	..	..	..	..	3	10	3	9
				Group X—Long Green type					
40–16	Konkan	21·7	16·9	10·5	13·3	4	9	5	5
40–31	do.	18·8	16·3	12·7	9·8	7	15	5	9
Local	do.	..	..	..	..	1	9	3	12

Remarks.—All the cultures with the exception of 32–8 put up satisfactory performance. Further individual selections were made and tested along with a few more during the next year.

During the year 1934–35, there were in all 48 cultures under study. There was severe cold during the month of January and many cultures suffered badly. Those that showed resistance and gave a satisfactory yield were studied. These were compared with their respective locals in the group. The performance in respect of their important characters is given in Table VIII.

TABLE VIII

Statement showing the performance of the cultures of Brinjal during 1934–35

Culture Nos.	Tract	Fruit length Mean (cms.)	No. of fruits per plant	Total yield lb.	Total yield oz.	Yield per plant lb.	Yield per plant oz.
		Group II. Long Purple					
Local	..	16·72	10·9	103	2½	4	2·0
25–32–22–30 ..	Deccan	**24·20**	**10·6**	**128**	**6½**	**4**	**15·0**
38–33–20–15 ..	Konkan	17·87	7·1	103	9½	3	9·2
38–33–20–16 ..	do.	**20·64**	**8·7**	**172**	**3½**	**5**	**11·8**
39–23–6–4	do.	**19·14**	**5·9**	**106**	**2½**	**4**	**3·9**
Duplicate 39–23–6–4	do.	17·55	6·2	71	15½	3	15·9
		Group III. Round Purple					
Local	..	10·92	9·4	166	1	5	9·9
10–3–20–21–5 ..	Gujerat	**12·13**	**7·9**	**136**	**13½**	**5**	**15·2**
15–12–9–18 ..	do.	11·46	10·1	157	14½	6	5·0
15–31–20–2 ..	do.	11·22	9·6	120	13½	6	0·6
15–31–20–5 ..	do.	**11·59**	**9·4**	**185**	**3½**	**6**	**13·7**
15–12–9–4	do.	11·21	10·4	270	15½	7	8·2
		Group IV. Gote Vangi					
Local	..	7·75	21·2	104	11	4	3·0
28–8–21–21 ..	Deccan	9·60	20·5	140	0	4	13·2
28–12–9–5	do.	8·55	8·9	128	6½	4	9·3
28–12–9–6	do.	10·06	13·4	131	13	5	7·8
28–21–24–3 ..	do.	9·34	20·2	192	11	7	2·1
28–21–24–10 ..	do.	**8·58**	**22·4**	**161**	**1**	**6**	**7·0**
32–7–3–7	do.	**9·25**	**17·6**	**166**	**11½**	**5**	**15·2**
32–10–3–3	do.	**8·87**	**15·1**	**121**	**14½**	**5**	**4·7**
32–10–15–4 ..	do.	9·54	19·6	202	14½	6	15·9
34–27–21–2 ..	Karnatak	9·89	16·4	175	4½	6	7·8
34–27–21–3 ..	do.	**9·20**	**20·4**	**180**	**9½**	**6**	**15·1**
		Groups VI, VII, VIII and XI. Dorli Vangi					
Local	Deccan	7·95	31·3	80	2½	3	1·2
8 C–1–12–7 ..	do.	7·79	27·6	69	4½	2	10·6
8 C–1–1–3–7 ..	do.	7·42	28·2	75	2½	3	6·2
8 C–1–1–3–13 ..	do.	9·00	14·5	149	13½	5	2·6
8 C–2–24–10–3 ..	do.	**5·62**	**50·2**	**112**	**6½**	**4**	**0**
23–3–24–3	do.	7·12	28·0	74	12½	2	10·6
23–3–24–4	do.	**6·37**	**32·6**	**117**	**4½**	**3**	**14·5**
8–3–24–2–3 ..	do.	**4·73**	**52·2**	**98**	**14½**	**3**	**4·7**
8–3–24–2–13 ..	do.	4·39	49·9	81	4½	2	14·8
30–18–23–2 ..	do.	**5·30**	**24·2**	**91**	**2½**	**3**	**4·1**
27–32–6–9	do.	7·95	23·7	178	10½	6	6·1
27–32–6–10 ..	do.	**7·05**	**27·5**	**141**	**13½**	**4**	**14·2**

The strains in bold figures were further selected. It was, however, decided that yield tests of the Dorli and the Gote selections being Poona brinjals should be taken up first for comparative yield trials. The other selections were maintained for subsequent trials. Only such selections as would conform to the ideal were selected.

C. Yield Tests

i. Gote selections.—It will be seen from Table VIII that some of the selections have put up very good performance and given high yields over the check. It was, therefore, decided to test the best selections by regular replicated trials (Latin Square). The Gote and the Dorli being the popular brinjals, the following Gote and Dorli selections, *viz.*, Gote 28–21, 32–7, 32–10 and 34–27 and Dorli 8–3, 8C-2, 23–3 and 27–32 were grown under their respective locals from 1935–39. The results of all the four Gote trials are summarised in the table below :—

(*a*) *Summary of results of yield trials of Gote selections*

Selection Nos. :—(A) 28–21, (B) 32–7, (C) 32–10, (D) 34–27, (E) Local.

TABLE IX

Mean acre yield in lbs.

Year	Selections					Z test P=0·05	Crt. dif. P=0·05	Remarks
	A	B	C	D	E			
1935–36	27347·2	24497·6	23038·4	23374·4	22932·8	..	..	.....
1936 37	14394·4	15072·0	13564·8	13344·8	9315·2	16·41	2410·48	B A C D E
1937–38	25607·2	23923·2	22296·0	20572·0	22382·4	2·41	3770·40	A B E C D
1938–39	6538·4	4929·6	4128·8	3844·8	4059·6	5·15	1499·152	A B C E D

In 1935–36 the test as a whole was not statistically significant. However, selection Nos. 28–21 (A) and 32–7 (B) showed increase in yield of 20 and 7 per cent. respectively over the local.

In 1936–37 the yield test as a whole was highly significant. Selections B, A, C and D were found superior in yield over the local.

In 1937–38 the result was significant on the whole. Among the strains, selection No. 28–21 (A) was found to be significantly better than selection No. 34–27 (D). The percentage superiority of A over the local was found to be 13·6.

In 1938–39, the test as a whole was significant. Strain No. 28–21 (A) is significantly better than the local (E) and other selections.

The combined analysis for 1935–36 and 1937–38 and also of 1936–37 and 1938–39 shows that the selections are superior to the local as can be seen from Tables X and XI.

(*b*) *Combined analysis of variognce of Gote selections.*—Some of the selections do not show any significance when the results are studied from year to year. An attempt was, therefore, made to see if a combined analysis would show any significance in the results. A combined analysis, however, can be considered to be strictly valid only if separate year's error variances do not differ significantly. By this test, the combined yields of the years (1) 1935–36 and 1937–38, (2) 1936–37 and 1938–39 were found to be significant. These results lead us to the general conclusions that some of the selections are significantly superior to the local as will be seen from the analysis given below :—

i. Combined analysis of Gote selections: 1935–36 and 1937–38

TABLE X

Due to	Deg. of freedom	Sum of squares	Mean square	Obs. F.	Crt. dif.
Rows	8	264575·8000	33071·9750		
Columns	8	22444·4114	2805·5514		
Varieties	4	20396·7673	5099·1918	*	386
Years	1	3209·1265	3209·1265		
Years × Varieties ..	4	1502·4598	375·6149		
Error	24	41970·1520	1748·7563		
TOTAL ..	49	354098·7170	..	..	..

* Sig. at P=0·05

Conclusions :—The experiment as a whole is significant. Selection No. 28–21 (A) has given 16 per cent. better yield over the local as can be seen from the variety totals given below :—

A	B	C	D	E
2455·02	2260·76	2113·45	2016·18	2115·59

The generalized conclusions are :

A B E C D

ii. Combined analysis of Gote selections: 1936–37 and 1938–39

TABLE XI

Due to	Deg. of freedom	Sum of squares	Mean square	Obs. F.	Crt. dif.
Rows	8	12677·0183	1584·6273		
Columns	8	8004·6965	1000·5871		
Varieties	4	13529·8136	3382·4534	**	
Years	1	139065·8870	139065·8870	**	182·5
Years × Varieties ..	4	6049·7752	1512·4438	**	
Error	24	5108·4539	212·8523		
TOTAL ..	49	184435·6445	..	..	..

** Sig. at P = 0·01

Conclusions : The variety totals are :—

A	B	C	D	E
1308·30	1250·08	1105·83	1074·38	835·80

The generalized conclusions are :—

$$\overline{A\ B}\ \underline{C\ D}\ \overline{E}$$

The following statement giving percentage increase or decrease from 1935–36 to 1938–39 shows that selection No. 28–21 gives 26% over the local.

TABLE XII

Strain Nos.	*Total yield per acre in lbs.				Total yield lbs.	Percentage over local
	1935–36 total area 1¼ gts.	1936–37 total area 2½ gts.	1937–38 total area 2½ gts.	1938–39 total area 2½ gts.		
28–21 (A)	27346·88	14394·08	25606·88	6538·72	73886·66	26·0
32–7 (B)	24497·60	15072·00	23923·36	4929·28	68422·24	17·0
32–10 (C)	23038·40	13564·48	22296·00	4128·80	63027·68	7·0
34–27 (D)	23373·76	13344·96	20572·00	3845·12	61135·84	4·0
Local (E)	22933·44	9314·88	22382·72	4057·92	58683·96	..

* The total yield is calculated from actual plot yields

i. Gote Selections

Gote selection No. 28–21 has a sweet taste and possesses less number of seeds and more pulp. It has an attractive colour and lustre and is much appreciated in the market. Being of uniformly medium size, it can be cooked like Dorli (whole fruit partially cut and stuffed with spices). This selection was given a trial on the Dharwar Farm for two years and it

is reported that it excelled all other varieties there. This selection, therefore is considered to be the suitable type of brinjal for this province.

ii. Dorli Selections

From Dorli varieties of brinjal, four selections, *viz.*, 8-3-24, 8C–2–24, 23–3–24 and 27–32–6 were made and in the year 1935–36 were replicated with the local on ¼ guntha plots by Latin Square method. In three replications there were many gaps due to death of plants and in other plots the plants did not put forth satisfactory growth. Due to these conditions, the results were badly vitiated and they have not been, therefore, represented here.

The yield tests of Dorli selections from 1936–39 are summarised in the following table.

(*a*) *Summary of results of yield trials of Dorli selections*

Selection Nos. (A) 8-3-24, (B) 8C–2–24, (C) 23–3–24, (D) 27–32–6, (E) Local.

TABLE XIII

Mean acre yield in lbs.

Year	Selections					Z test P = 0·05	Crt. dif. P = 0·05	Remarks
	A	B	C	D	E			
1935–36	..	..	..	..	..	..	..	..
1936–37	8356·8	7732·8	6599·2	8120·8	7246·4	..	..	..
1937–38	13056·8	11463·2	10316·8	11684·0	9843·2	..	..	..
1938–39	5133·6	4408·0	4498·4	6138·4	4026·4	..	..	..

The results when analysed individually from year to year do not give any significant result. In 1936–37, however, selection Nos. 27–32–6 and 8–3–24 give 19·11 and 11·88 per cent. increase respectively over the local.

In 1937–38, selection Nos. 8–3–24, 27–32–6 and 8C–2–24 showed, 30 36, 18·18 and 61·22 per cent. superiority over the local.

In 1938–39 selection Nos. 27–32–6, 8–3–24 and 8C–2–24 exceeded the local by 47·09, 26·16 and 12·73 per cent. respectively.

The combined analysis of variance of Dorli selections for all the three years, however, shows clear significance in favour of Nos. (D) 27–32–6 and (A) 8–3–24 over the others as can be seen from the following table.

(*b*) *Combined analysis of Dorli Selections* 1936–37 to 1938–39 (3 years)

TABLE XIV

Rows, varieties and years are compared against the interaction " varieties × years ", using the latter as ' error ' giving 7·65 as variance ratio.

Due to	Deg. of freedom	Sum of squares	Mean square	Obs. F.	Crt. dif.
Rows	12	79172·6897	6597·7241	**	
Columns	12	12246·9971	1020·5831	**	
Varieties	4	7698·8307	1924·7077	**	291
Years	2	81940·2192	40970·1096	**	
Years × Varieties ..	8	2012·6019	251·5753		
Error	36	19338·9692	537·1936		
TOTAL ..	74	202410·3078			

** Sig. at P= 0·01

Conclusions :—

The variety totals are :—

A	B	C	D	E
1659·19	1481·51	1319·64	1671·43	1332·25

The generalized conclusions are:—

D A B E C

These results can be regarded as holding good over a period of years of which the three experimental years can be regarded as a random sample.

In the statement given below the percentage increase or decrease from 1935–36 to 1938–39 is given. It will be seen that selection No. 27–32–6 (D) has given the highest percentage of yield over the local. Selection No. 8–3–24 (A) comes next with regard to yield.

TABLE XV

Statement showing percentage of increase or decrease in yield of Dorli selections from 1935–36 *to* 1938–39

Strain Nos.	*Total yield per acre in lbs.				Total yield lbs.	Percentage over local
	1935–36 total area 1¼ gts.	1936–37 total area 2½ gts.	1937–38 total area 2½ gts.	1938–39 total area 2½ gts.		
8–3–24 (A)	..	8356·96	13056·80	5133·28	26547·04	24·54
8 C–2–24 (B)	..	7732·80	11463·20	4408·16	23704·16	11·20
23–3–24 (C)	..	6599·04	10316·64	4198·56	21114·24	– 0·94
27–32–6 (D)	..	8920·80	11684·00	6138·08	26742·88	25·46
Local (E)	..	7446·56	9843·20	4026·24	21316·00	..

* The total yield is calculated from actual plot yields

Dorli Selections.—The Dorli variety is the most seedy and the smallest among brinjals. Selection No. 27–32–6, however, bears bigger sized fruit with less number of seeds. It is tasty and has an attractive colour. Further, it is an early type which can be put on the market by about a fortnight earlier. This is also considered to be suitable for the Deccan tract.

Summary

Very little work has been done in India on the improvement of the brinjal crop by selection. In the Province of Bombay this work was started in the rabi season of 1931 and was financed by the Sir Sassoon David Trust Fund. Various collections of samples of brinjals cultivated in different tracts of the Bombay Province were made and studied in detail for their fruit and plant characters. As a result of these studies the various brinjal types have been grouped under 11 classes with several horticultural forms in each. Gammie and Mollison have grouped all brinjals under three classes only.

Promising selections have been isolated and their behaviour has been further studied for their purity and other characters. Selections from the Gote and the Dorli varieties only were compared with their respective local varieties, in the Fisher's randomization method. These trials were made for four seasons. Gote selection No. 24-21 gave significant results during the two years of trial. This strain bears fruits of uniform medium size and has attractive colour and lustre. As it is less seedy and has more and tasty pulp it is highly appreciated in the market. Dorli selection No. 27–32 gives appreciably better yield than any other Dorli strain or the local. This is a promising selection. Dorli brinjals are the smallest and the most seedy among all brinjal types known. This selection, however, bears bigger-sized fruits and has less seeds. It has an attractive colour and is very tasty. It flowers earlier by about a fortnight and is in great demand in the market.

Further work, as chalked out in the Scheme, had to be discontinued for want of funds. The selections are, however, maintained in their pure condition and are being distributed for trial all over the districts.

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Appendices

APPENDIX I

Acreage under brinjal crop during 1937–38

	Acres	Percentage for the district
Gujerat—		
1. Ahmedabad	989	
2. Kaira	688	
3. Broach and Panchmahals ..	768	
4. Surat	1,145	
TOTAL ..	3,590	21·0
Deccan—		
1. West Khandesh	500	
2. East Khandesh	1,051	
3. Nasik	845	
4. Ahmednagar	909	
5. Poona	1,758	
6. Sholapur	1,224	
7. Satara	1,499	
TOTAL ..	7,786	45·6
Karnatak—		
1. Belgaum	1,331	
2. Bijapur	1,049	
3. Dharwar	1,775	
TOTAL ..	4,155	24·3
Konkan—		
1. Thana	662	
2. Bombay Suburban ..	85	
3. Kolaba	220	
4. Ratnagiri	455	
5. Kanara	80	
TOTAL ..	1,542	9·1

Grand total for the Province of Bombay : 17,073 acres

APPENDIX II

Brinjals—Statement showing the history of the original samples

Ser. Nos.	Original Nos.	Source, etc.	Fruit length cms.	Diameter cms.	Local name	Nature of fruit
			Foreign types			
1	3	Foreign	16·4	15·5	Black Beauty	Purple, conical
2	4	do.	10·1	4·8	Early Dwarf	Purple, oval, bigger than an egg
3	5	do.	17·5	8·5	Early Long Purple	Purple, oval
			Deccan types			
4	8	Poona, Nasik and Sinnar	5·2	3·1	Dorli	Small, round
5	8 C	do.	7·4	5·1	do.	do.
6	23	Nasik	6·7	3·5	do.	do.
7	24	do.	20·6	4·5	Bengali lamb hiravi	Long, green
8	25	do.	21·0	4·7	Bengali lamb kali	Long, black
9	26	Poona	8·3	3·7	Gavati	Thorny fruit
10	27	do.	7·8	6·0	Dorli	Small, oval, thorny
11	28	do.	10·0	6·3	Gote vangi	Round, thorny
12	29	do.	11·0	5·8	Mothi binkateri	Big, thornless
13	30	do.	4·8	4·0	Lahan kate vangi	Small, thorny
14	31	Ahmednagar	7·5	3·7	Gavati	Local
15	32	Sholapur	10·0	7·3	Lamb gole gote	Large, round gote
16	33	do.	10·0	7·2	Lambdi lahan	Small, long
17	18	E. Khandesh	8·5	7·5	Gavati	Local
18	19	do.	5·5	3·8	Kali vangi	..
19	20	do.	7·7	4·0	Lahan kateri vangi	Small, early, thorny
20	21	do.	19·0	5·1	Lamb kali	Long, black
21	22	do.	7·7	5·4	Lahan gol	Small, round, striped, thorny
			Karnatak types			
22	11	Belgaum	7·4	5·1	Krishna-tir	Round, green
23	34	do.	10·6	8·5	Malvanki	Round, thorny
24	35	Dharwar	10·0	6·0	Lamb kali	Long, black
25	36	do.	12·7	6·9	Lahan gol	Small, round
26	37	Bijapur	11·9	11·8	Gavati	Local
			Gujerat types			
27	10	Surat	10·5	5·6	Surti	Round, purple
28	13	Ahmedabad	14·2	5·2	..	Long, black and thick
29	14	Kaira	18·0	5·6	Surti	Round, purple
30	15	Broach	11·4	8·3	..	Big, round with less number of seeds
31	16	Surat	4·4	3·3	Surti Ravaya	Small type
			Konkan types			
32	17	Thana	6·6	5·2	..	Thin rind, less seeds and sweet
33	38	do.	17·5	5·9	Pandhri vel vangi	Long
34	39	do.	23·0	8·5	Kali vel vangi	Long, black
35	40	do.	16·9	7·6	Gol kabri	Long, green
36	41	do.	23·1	6·3	Lamb jabhari	Long, purple,
37	42	do.	16·0	4·5	Vel vangi	do.
38	43	do.	15·3	5·4	Kas vangi	Long, green
39	44	Kolaba	14·8	9·0	..	Long variety, less seeds
40	45	do.	7·3	8·2	Mushakdanti	Sweet, round, purple
41	46	Ratnagiri	17·0	6·0	..	Long, purple
42	47	do.	18·8	5·8	..	Long, black
43	48	do.	16·7	6·1	..	Long, mixed colour
44	49	do.	8·8	7·4	..	Long, black
45	50	do.	8·4	7·8	..	Red, round

Added two exotic varieties later

APPENDIX III

Information in respect of the plot and the method of cultivation followed in growing brinjal selections

Season and Year.—The Gote and the Dorli selections were grown as rabi crop under irrigation throughout the experimental work. The sowing was done in the first week of September and the seedlings were transplanted after 6 weeks.

Nature of comparison.—Gote selections Nos. 28-21, 32-7, 32-10 and 34-27 were compared with the Gote local and the Dorli selections Nos. 8-3-24, 8C-2-24, 23-3-24, and 27-32-6 were compared with the Dorli local in Latin Square.

Previous cropping.—Jowar was invariably raised for fodder before planting the rabi brinjal crop.

Nature of plot.—The plot is on river side and has loose soil all over.

No. of replications.—The number of replications in each trial is 5. Each sub-plot measured ¼ guntha* in the first year and ½ guntha in all subsquent years.

Cultivation.—The seedlings were planted at 3 ft. apart on ridges 3 ft. apart. The plot was regularly irrigated at 8-10 days interval, manured with 15 cartloads of F. Y. M. and regularly weeded. The plants were earthed up and the furrows for irrigation were frequently repaired.

General.—All weather changes and condition of crop from season to season was recorded and taken note of.

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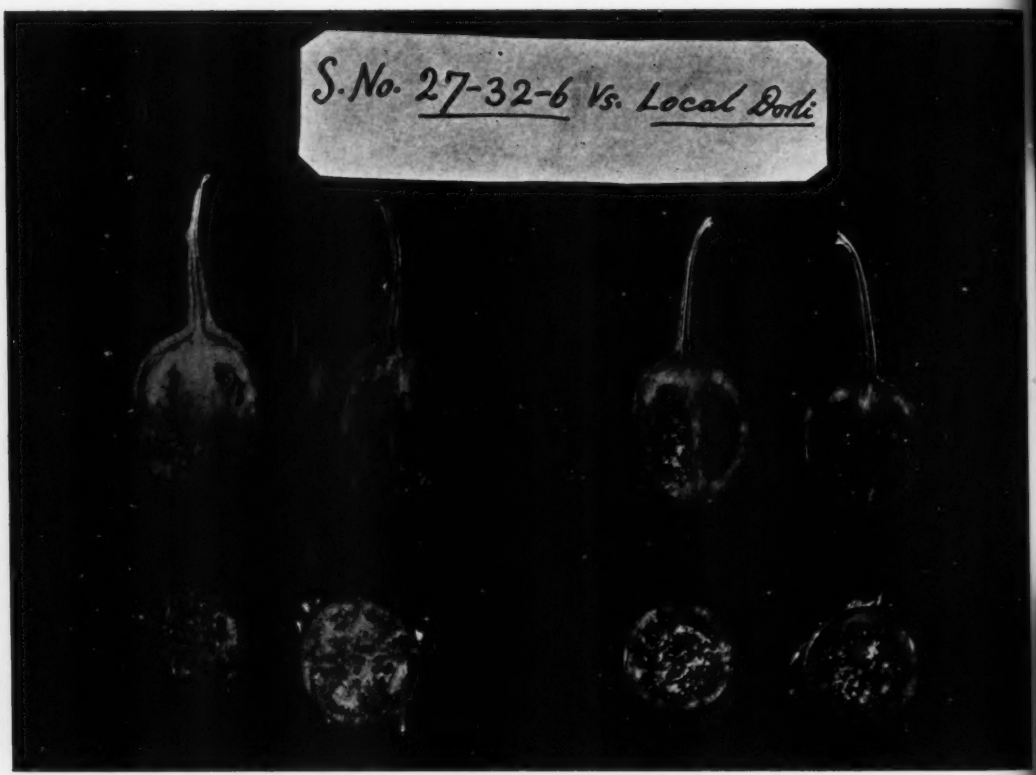
* *Note.*—One guntha is equivalent to 1/40 of an acre.

368-42—Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore

Fruits of No. 28-21 selection are of uniform size and contain less seeds and more pulp as compared with Gote local

Left—Selection on 28-21
Right—Local



Fruits of No. 27-32-6 selection are bigger in size and less seedy as compared with the Local Dorli

Left—Selection No. 27-32-6 Dorli

Right—Local

ERRATUM

Vol. XVI, No. 1, Plate IV, Fig. 13 reverted.

ON A PERENNIAL FORM OF *SCYTONEMA* (*S. OCELLATUM* LYNGB. FORMA *MINOR* BHARADWAJA), AND ITS AUTECOLOGY

By Rama Nagina Singh, M.Sc.

(Department of Botany, Benares Hindu University)

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Introduction

In contrast to the large amount of synecological work that has been published during the last two or three decades, our knowledge of autecology, even of the commonest species of Myxophyceæ, is still very meagre, and the time has now come when the conditions which determine the occurrence, growth, reproduction and perennation of the most characteristic species of the diverse types of plant communities in nature should be studied in detail, if any real progress in synecology is to be made. The present work, carried out from January 1939 to June 1940, was therefore done with a view to gaining some knowledge of the autecology of *Scytonema ocellatum* Lyngb. forma *minor* Bharadwaja (*cf.* Bharadwaja, 1934), especially in connection with its soil requirements.

The alga was, for the first time, observed growing on the lawns of the Botanical Garden of the Benares Hindu University, in the middle of January 1939, just after a slight shower of rain due to winter monsoon. Since then it has been found to inhabit other places also. It forms a woolly tufted growth of an ash-brown colour. The growth is pure and isolated from other algæ, thus constituting a natural plant community by itself.

The stratum when first appearing, is almost of the size of a pin-head ; later it becomes wider and exhibits erect tufts. Its growth is at first regular as evidenced by its spherical shape, but this appearance is maintained only till it is 4 cm. in diameter, after which the stratum becomes extended irregularly. This irregularity in outline is increased by the coalescence of several small colonies into one and also by the growth of the phanerogamic vegetation which confines the alga to their interstices. In course of time the expanse of the thallus becomes so large that the whole ground appears to be covered with a thick velvetty and gelatinous growth. As the ground

loses water by evaporation the stratum gets dried up to form a greyish-black papery incrustation. In this condition it persists for a considerably long period without getting mixed up with the soil. It is then blown about by wind from place to place. At places where the necessary moisture is available throughout the year the alga is perennial, but at those where it forms dry flakes the alga regains its activity to grow into fresh thalli soon after the soil is irrigated. During the rainy season, however, the alga is a very prominent participant in the vegetation of the country. Its occurrence is by no means insignificant even in the winter season, especially in December and January, when the dew formation is intense. Soil moisture is therefore the chief ecological factor controlling the growth and development of the plant in question.

Morphological Behaviour of the Alga

The filaments constituting the tufted growth of the alga are 2-5 mm. in length. During vegetative condition, they are free, but when perennating they get entangled with each other to some extent. They are at first tapering but afterwards of uniform thickness throughout. The trichomes also change their colour from bluish-green to dark-brown which shade they generally exhibit. They are delicate and enclosed in a slightly lamellose sheath, which is hyaline or pale-yellow in young filaments but brown or dark-brown in old ones. The lamellose nature of the sheath has been observed only in old filaments after treatment with 1% aqueous solution of methylene blue. The septa are at first indistinct, but they become prominent as the trichome becomes old. The filaments vary in thickness, the breadth of the young ones being 9-11·8 μ and that of the old ones 11·8–14·7 μ. There are both single and geminate pseudobranches arising by the side of dead cells and two-pored heterocysts. The vegetative cells, having coarsely granular contents, are almost quadrate or a bit shorter than broad, and measure 8·8-11·0 μ in diameter. The sheath is uniformly thick and measures 1·5-4·0 μ. It is quite firm and retains its cylindrical shape even when a number of cells of the trichome die or when hormogones have escaped from the sheath and left it empty.

Heterocysts are found only in older filaments, being absent from very young ones. Ordinarily there is a single median heterocyst, but two, three or more may also occur at intervals along the length of the filament. The filaments do not bulge opposite the heterocysts. The heterocysts are pale-yellow in colour, the contents being at first granular but later homogeneous. They are usually quadrate, but rarely cylindrical ones are also met with, being 5·5-8·8 μ broad and 6·0-8·8 μ long.

Multiplication is mostly effected by means of short hormogones, which are formed by the dying away of certain cells here and there and also by the secretion of an intercellular substance of dark green colour between two adjoining cells as has been known in most of the Hormogonales (Ghose, 1920; Bharadwaja, 1928, 1933, 1940). The hormogones are both short and long, consisting of one or more cells.

Perennation in this alga, during the unfavourable conditions, is accomplished either by the entire filaments or by hormogones remaining dormant inside the thick brown sheaths (*cf.* Bharadwaja, 1933, 1940). On the recurrence of favourable conditions the hormogones slowly emerge from the old sheaths, at the same time secreting new hyaline sheaths. They then grow into mature filaments by repeated cell-division. In some cases parts of the old parent sheath still enclose the new filament. No spores have been observed in this alga.

Edaphic Factors Determining Growth and Occurrence of the Plant

Moisture Content.—Although it is generally recognised that moisture is an important factor in the development of soil algæ, very little direct experimental evidence is available concerning this effect. Esmarch (1914) attributed the greater number of Cyanophyceæ in cultivated soils than in uncultivated ones to the fact that cultivation by improving the physical structure of the soil, brought about better nutritive and moisture conditions for algal growth. Bristol (1919, 1920) studied the effect of desiccation upon algæ. Soils obtained from arable lands as well as from old gardens were spread out to dry in a warm room for at least a month. Despite the long period of desiccation, the soils were found to contain 64 species and varieties of algæ consisting of 24 species of Cyanophyceæ, 20 species of Chlorophyceæ and 20 species of Bacillariophyceæ (diatoms). Bristol also reported that she was able to isolate a species of blue-green alga, 4 grass-green algæ and one diatom from dry soil stored for about 40 years. Fritsch (1922, 1936) pointed out that a striking characteristic of terrestrial algæ is the capacity of the ordinary vegetative cells, without any marked change and without any special thickening of the walls, to withstand drought. Moreover, the change from the resting to the active condition was found to occur in a very short time, apparently because the terrestrial algæ required only small amounts of moisture to replace that lost by the protoplast in drying. These investigations indicate that many soil algæ are resistant to desiccation, but they give no idea as to how algæ respond to varying moisture conditions. That the moisture content of the soil is an important factor in algal development is amply shown by the general observation that the soil is covered with a prolific

growth of these plants in the rainy season. Further englihtenment on the subject is given by the following experiments and observations made from the field material in various seasons.

Soil blocks, with the dried up papery incrustation of *Scytonema* were sampled from the field, in the month of May, 1939, without the least disturbance whatsoever of the surface layer. More or less similar samples were taken in each of the ten glass troughs. One trough was left untreated; the moisture levels of the soil in the remaining nine troughs were adjusted to 5, 7, 8, 10, 20, 40, 60, 80 and 100% of the moisture-holding capacity of the soil, which is 45·3. The troughs were covered with glass covers to keep out atmospheric contaminants and incubated in the laboratory at room temperature and in diffused light obtained from a north window. Calculated amounts of water were added each day to compensate for losses of moisture due to evaporation.

Within two days, the dried up papery surface incrustation of the alga regained fresh growth and the stratum became visible on the soils brought to 7, 8, 10, 20, 40, 60 and 80% of the moisture holding capacity. The cultures having 10, 20 and 40% moisture appeared to have the most abundant algal growth; and the rest least. There was, however, no apparent algal growth on the surface of the culture brought to 5% moisture level. After a week's incubation, the relative growth in the differently treated samples was the same as after two days, but it was more abundant. After ten days' incubation, the surface of the soil in each culture to a depth of 1 cm. was analysed for the number of algæ (Table I). The method recommended by Bristol Roach (1926) for counting the number of algæ in the soil was employed. This method was found to be extremely tedious and cumbersome, because of the large number of dilutions required for each count. In an ecologicl work, like the one treated here, it was thought desirable to adopt some rapid method of computation so that it might be possible to take a number of readings in short time. The measurement of the relative growth of the cultures was, therefore, considered quite feasible. For this purpose 5 sq. mm. surface growth was scraped off by means of a razor blade and the number of filaments contained therein was counted under the field view of the low power of the microscope. Only such filaments were included as were found to be in normal and active growth. The frequencies were expressed by symbols as given below. The numerical values given to these symbols, though

Prolific	P	500	Slight	S	30
Abundant	A	350	Insignificant	I	10
Good	G	200	..	..	..
Fair	F	75	Nil	N	0·5

arbitrary, approximately correspond with the number of individuals observed and give satisfactory idea for the relative growth of the alga in the different cultures. It may be pointed out here that all numerical values for the alga given in the subsequent pages have been made by adopting the method outlined above.

TABLE I

Effect of Soil Moisture upon Growth

Saturation of soil %	Algal Growth	No. of filament per 5 sq. mm. of Soil
5	I	15
7	S	30
8	G	150
10	P	400
20	P	500
40	P	510
60	A	420
80	G	300
100	S	50
Control (4%)	N	..

The experimental results obtained in Table I were checked by observations made on field material. The soil samples were taken in the different months of the year at fortnightly intervals, their moisture content and number of the alga were calculated according to the method given previously. The results represent the mean of the number of determinations in each month for 25 samples taken at random from widely separated localities (*cf.* Fisher, 1934), and are tabulated as in the following table.

TABLE II

Moisture content of soil, Rainfall and number of filaments of the alga in different months of the year, 1939–40

Months	Rainfall in inches	Saturation of Soil %	No. of filaments pe. 5 sq. mm. of Soil
March ..	..	1·2	..
April ..	0·01	1·0	..
May ..	..	0·8	..
June ..	4·56	1·4	..
July ..	10·37	50·5	510
August ..	6·03	60·0	250
September ..	5·66	70·5	115
October ..	0·21	35·2	450
November ..	..	20·5	150
December ..	..	22·5	470
January ..	0·24	25·0	500
February ..	0·58	8·5	75

The results presented in the previous tables are graphically plotted in Figs. 1 and 2. It is seen from Fig. 1, that in cultures the alga attains the maximum growth, as determined by the increase in its number, with increasing moisture upto 20% of the water-holding capacity of the soil, and it continues to be the same upto 40%. There is then an abrupt fall until finally the number becomes considerably less at complete saturation. The same appears to be true under field conditions because the nature of the two curves are more or less similar. This indifference of the alga to rising moisture levels in the soil is due perhaps to the fact that high saturation brings about anaerobic and inimical conditions which upset the respiratory balance of the plant. It shows, therefore, from the above findings that the optimum moisture range for the normal growth of the alga lies between 10 and 20% of moisture of the water-holding capacity of soil.

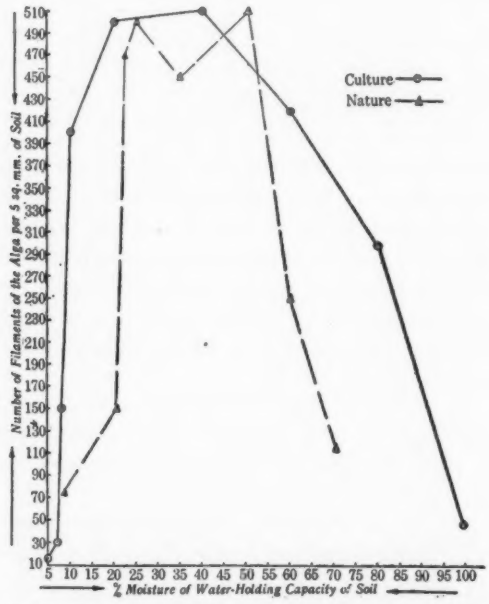

FIG. 1. Effect of Moisture Content of Soil on the Growth of *Scytonema ocellatum* Lyngb. forma *minor* Bharadwaja.
Continuous curve representing cultural behaviour, broken lines field observations.

Fig. 2 gives data for the seasonal distribution of the alga and the rainfall in different months of the year under consideration. It is observed from this figure that in the month of July the alga determines the dominant feature of the vegetation of the country; but, at intervals, when the soil is

waterlogged for days together due to continuous rainfall, the growth of the plant is very much impeded, almost to its complete extinction. This behaviour of the plant becomes more evident in the months of August and September when the soil is greatly saturated with moisture. It is recognised, therefore, that the growth of the plant is controlled by intermittent showers. Any tendency towards waterlogging the soil results in the total retardation of the growth of the plant. During the first half of October there is again a sudden rise in the number of the alga and a similar fall in the month of November because of the high temperature which causes intense evaporation of water from the soil and also due to the absence of rainfall. In the months of December and January, however, the plant regains its former luxuriance due to heavy dew formation, which makes the surface soil quite wet. During the summer season the alga is unable to make any growth.

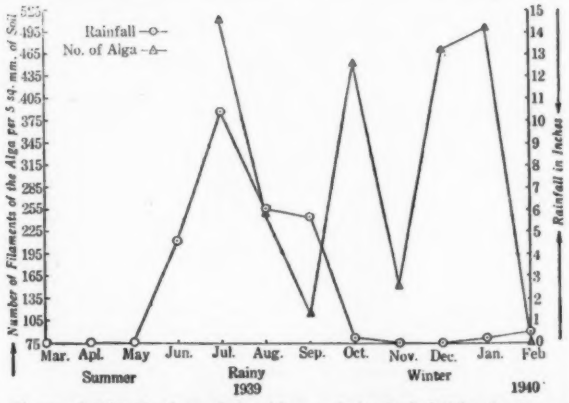

FIG. 2. Seasonal Distribution of the Alga and the Rainfall in the Year 1939-40. Circles represent the rainfall ; triangles are for the alga.

Organic Contents.—As quoted by Petersen (1935), Treub (1888) observed the growth of blue-green algæ (*Tolypothrix*, *Anabæna*, *Symploca*, and three species of *Lyngbya*) on the barren mineral layer after the destruction of soil and vegetation of Krakatoa by volcanic eruption, and considered them to be the first to colonize the land. According to the same author (1935), Græbner (1895) listed the species of soil algæ from the heaths of northern Germany, and found them as the first immigrants on new soil, causing the first formation of humus in the poor soil. Fritsch (1907, 1910) discussed the importance of the blue-green algæ as the first colonists on new soil. Like several previous workers, he (1936) also pointed out that the inclusion of their (soil algæ) substance in the soil, whether direct or indirect, must enrich it in organic matter. But, quantitative data are, however, lacking

as regards the behaviour of algæ to different levels of organic matter in the soil.

Besides the growth of the alga on the lawns of the Botanical Garden, as has been mentioned in the preceding pages, it was also found to grow in the flower-beds in the garden, which were lying fallow at the time of observation. These beds were previously given a heavy dose of farmyard manure. Advantage of the same was therefore taken to study the effect of organic content of the soil on the growth of *Scytonema ocellatum* Lyngb. forma *minor* Bharadwaja. Soil samples were taken from the lawns as well as from the flower-beds; their organic and nitrogen content and the number of the alga were determined. The results are given in the following tables (Tables III & IV).

TABLE III

Organic and nitrogen content, C/N ratio and number of filaments of the alga in the soil samples collected from the lawns uncultivated and barren lands

Organic Matter % of dry wt.	Nitrogen % of dry wt.	C/N	No. of filaments of the Alga per 5 sq. mm. of Soil
0·6953	0·0434	8·01	285
0·6736	0·0413	8·15	310
0·7325	0·0456	8·03	290
0·7173	0·0435	8·24	312
0·6631	0·0394	8·41	320
0·7214	0·0448	8·05	300
0·7315	0·0473	7·73	250
0·0187	0·0457	7·86	266
0·6845	0·0417	8·20	312
0·6816	0·0435	7·83	278
0·7406	0·0482	7·68	250
0·6907	0·0418	8·26	312

TABLE IV

Organic and nitrogen content, C/N ratio and number of filaments of the alga in the soil samples collected from the flower-beds

Organic Matter % of dry wt.	Nitrogen % of dry wt.	C/N	No. of filaments of the alga per 5 sq. mm. of soil
1·1740	0·0588	10·91	440
0·9960	0·0485	10·26	368
1·1833	0·0603	9·81	340
1·2045	0·0632	10·32	374
1·2032	0·0613	9·81	345
0·9854	0·0455	10·82	420
0·9957	0·0493	10·09	355
0·9934	0·0474	10·47	385
1·2141	0·0684	8·87	325

The results presented in the above tables (Tables III and IV) show clearly that the plant behaves indifferently to organic content of the substratum, which is in full agreement with the view, very often expressed, regarding the nature of the blue-green algæ as colonists. On the other hand it is seen from Fig. 3 where the C/N ratios are plotted against the number of the alga, that it is the balance between carbon and nitrogen that determines the growth of the plant in question. At lower C/N values the alga makes little growth but, as the C/N ratio increases it reaches its maximum number.

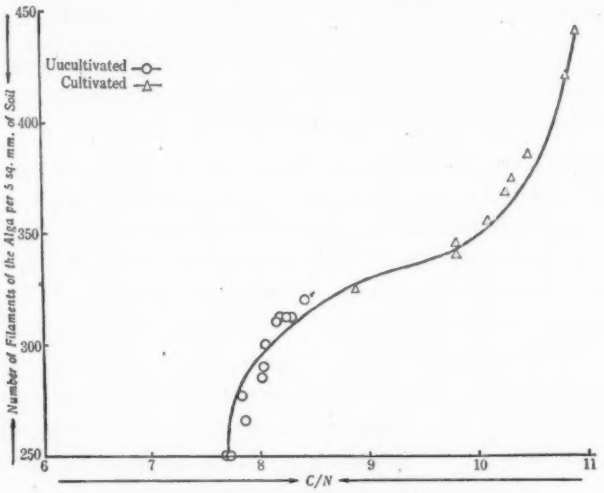

FIG. 3. C/N ratio in relation to the number of filaments of the alga. Circles represent data for soil samples collected from lawns, uncultivated and barren lands; triangles those from flower-beds.

Hydrogen-ion Concentration.—The next factor that has been taken into consideration is the pH value of the soil. Hydrogen-ion concentration plays a very important rôle in controlling the metabolic activities of the plants. The physiological action of the free hydrogen-ions is considerable and a slight change in the hydrogen-ion concentration may have a critical effect. Petersen (1935) has reported that the algal flora in acid soils is different from that found in alkaline or neutral soils. Allison and his co-workers (1937) have reported that the nitrogen-fixing blue-greens thrive best in nearly neutral or alkaline soils. They also maintain that *Nostoc* may be able to continue to multiply at the surface even in acid soils, because by growing in colonies and constantly removing CO_2 from the soil during photosynthesis it may increase the pH locally. De (1939) found a good

algal growth, mainly of the Myxophyceæ in certain of the cultures of paddy field soils from Faridpur, having a high pH.

The pH values of a number of soil samples characterising the growth of the alga were determined in quinhydrone saturated soil suspensions of 1 : 3 soil and water, the potential at a bright platinum electrode being measured against that of a saturated potassium chloride solution and a saturated calomel electrode. The details, as given by Wright (1939) and also by Loomis and Shull (1937), were followed throughout the work. pH was calculated from the formula, pH $7{\cdot}8 - \frac{potential\ difference}{0{\cdot}058}$ at 20°C.; but in actual practice pH was read directly from the potential difference readings plotted upon a graph for that purpose in accordance with the formula given above. The data thus obtained are recorded in Tables V and VI.

A review of the results given in the above-mentioned tables shows clearly that slight changes in the hydrogen-ion concentration of the substratum do not have any marked influence upon the growth of the alga. A neutral or nearly alkaline medium is decidedly preferable for the plant. The pH range varies between 6·4 and 7·4. It is also found that at higher pH values the number of the alga is greater provided the redox potential is fairly high.

An attempt to find out optimum pH value for the alga has given to disappointing results. Fig. 4 shows that there is no fixed optimum for the plant and it varies both on the acidic and alkaline sides of the pH scale.

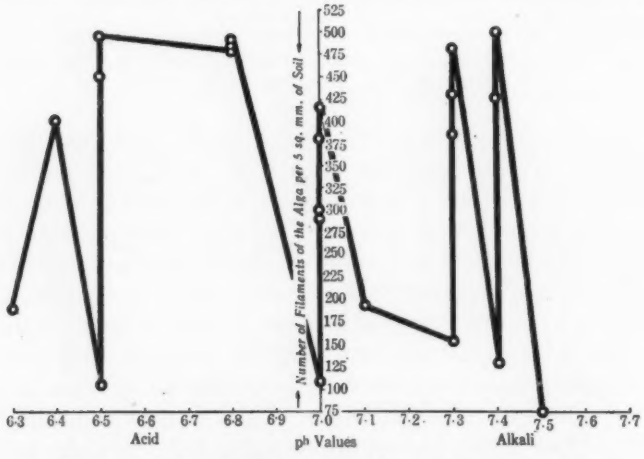

FIG. 4. Graph representing the optima of pH for the alga.

The bimodal curves of Salisbury and others and the unimodal curve of Pearsall (1926) and Volk (1931) for the higher plants, as quoted by Braun-Blanquet (1932), cannot be applied to lower plants as the one considered here. The pH optimum for *Scytonema ocellatum* Lyngb. forma *minor* Bharadwaja, examined in natural habitats, results in a many-peaked curve (Fig. 4). This indifference of the alga to changes in the pH of the medium lends further support to the prevalent view of the colonising nature of the blue-greens.

Oxidation-reduction potential.—The importance of the effect of oxidation-reduction potential on plant growth is drawing the attention of a number of workers (*e.g.*, Bradfield and his co-workers, 1934; Misra, 1938; Volk, 1939) at the present time. Its role in delimiting natural plant communities (Woodland, Mooreland and Bog Communities) in northern England has been emphasized by Pearsall (1938). But, all these studies have been carried on with higher plants, and there is practically no work in record on the effect of redox potential on the growth of algæ, especially the blue-greens that have often been considered as indicators of soil conditions.

The procedure of the work adopted was as follows. The electrical potential of the soil was measured, and was assumed to represent the oxidation-reduction potential, with higher values indicating greater intensity of oxidation. For this a bright clean platinum electrode was inserted into the soil sample collected from the fields. This electrode was connected with a saturated potassium chloride-calomel electrode for reference as the standard electrode. The circuit of the cell was made complete through a Cambridge portable potentiometer fitted with a tap key. An opposite standardised current ran through the instrument from 1 V. dry cell. The potential difference between the soil system and the standard electrode was then measured in volts by adjusting the resistance scale to null point. The final value for E_h was obtained by adding 0·25 V. to these readings since it is the difference of potential between the saturated calomel electrode and a normal hydrogen electrode at 20° C. The readings are given as E_h adjusted to pH 7 and called E_7. The adjustment is made on the assumption that an increase of unit pH will involve a decrease of 58 m.V. in E_h. But, as Pearsall (1938) remarks this may be too low.

Soil samples, possessing the characteristic growth of the alga in question, were investigated for the redox potential. Most of the readings were taken in the field and in all cases the potential was found to be quite steady after 20 minutes. The samples were also analysed for the number of

individuals of the alga by the method described previously. Tables V and VI summarise the observations made.

A consideration of the data presented in the above-mentioned tables and illustrated by Fig. 5 shows that the E_7 range for the alga varies between 410 and 465 m.V. At lower E_7 values the growth is very much retarded. The magnitudes of the potentials, E_7, shows that all these soils are oxidising in nature. This is also proved by the absence of ferrous iron with the thiocyanate test, a detailed consideration of which is postponed to a later stage. Cultures with their high water content register very low potentials, which fluctuate considerably in the beginning but after a week become quite steady. The observations given in Table VI represent those that were taken after the potential became constant. As the moisture content of the samples increase the potentials decrease.

TABLE V

Potentials and the growth of the alga in soil samples freshly collected from the fields

No. of Samples	pH	$E_{\frac{1}{2}}$ (m.V.)	E_7 (m.V.)	No. of filaments of the Alga per 5 sq. mm. of soil
1	7·4	427	450	500
4	7·3	395	412	480
7	6·5	439	410	450
10	7·0	395	395	380
11	6·8	458	446	484
19	6·2	422	376	136
21	7·3	358	375	154
22	6·3	418	378	190
24	7·1	390	396	194
28	7·4	395	418	424
32	7·3	380	397	385
33	6·8	430	418	492
39	6·5	450	421	495
45	6·5	398	369	105
46	7·5	330	359	75
67	7·0	390	390	108
78	7·0	398	398	290
82	7·3	398	415	430
84	7·4	375	398	130
85	7·0	398	398	300
88	6·8	436	424	478
90	6·4	435	400	400
94	7·0	412	412	415

There is therefore an inverse proportionality between the two values (*cf.* Pearsall, 1938). High moisture levels in the soil reduce aeration and consequently there is a fall in the oxidation intensity.

TABLE VI

Potentials in relation to the number of the alga in the cultures

Saturation of Soil %	pH	E_h (m.V.)	E_7 (m.V.)	No. of filaments of the alga per 5 sq. mm. of soil
4 (Control) ..	6·8	484	495	..
5 ..	6·8	475	486	15
7 ..	6·8	464	475	30
8 ..	7·0	470	470	100
10 ..	6·5	494	465	400
20 ..	6·8	436	425	500
40 ..	7·0	410	410	510
60 ..	7·0	398	398	420
80 ..	7·2	344	355	300
100 ..	6·8	304	315	50

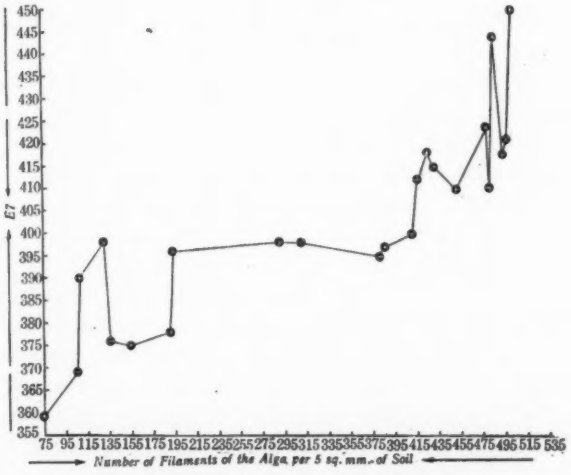

FIG. 5. Potentials in relation to the growth of the alga

Ammonium-Thiocyanate Test and Base-deficiency.—Determination of exchangeable bases is usually a lengthy process. In an extensive ecological work therefore a field test showing the balance between exchangeable cations like $Ca^{\cdot\cdot}$ and $K^{\cdot}$ and $Fe^{\cdot\cdot\cdot}$ and $Al^{\cdot\cdot\cdot}$ would be undoubtedly very useful. As a test for replaceable ferric and ferrous iron the soil is shaken with approximately twice its volume of saturated alcoholic ammonium thiocyanate and was then allowed to stand for 5–10 minutes till the solids had settled down, leaving a clear supernatant layer, the colour of which is recorded. One

drop of H_2O_2 (10 vol.) is added and any intensification of the colour noted (*cf.* Misra, 1938). The shades of the colour are recorded on an arbitrary scale (0–4) as, for example, 2/3, the former figure indicating the original colour produced by the free ferric ions, and the latter figure signifying the colour after the addition of hydrogen peroxide which oxidises any ferrous iron to ferric. This difference in colour is a measure of replaceable ferrous iron. It has already been noted that the measurement of the redox potential factor for determining the chemical state of the substratum with regard to oxidised or reduced substances present in it, is very essential in delimiting algal growth. Comber's test (1920) as modified by Misra (1938) answers the same purpose.

TABLE VII

Results of ammonium-thiocyanate test

No. of Sample	Comber's Colour		Reductivity
	Without H_2O_2	With H_2O_2	
1	3	3	..
7	2	2	..
32	1	1	..
39	3	3	..
46	1	2	1
82	2	2	..
85	1	1	..
88	4	4	..
94	3	3	..

Data about a few representative samples as obtained with the modified Comber's test are given in Table VII, from where it may be noted that most of the soil samples are oxidising in behaviour and therefore lack the reductivity test. It may be concluded therefore that the soils characterising the growth of the plant have high oxidation intensity.

Diphenylamine Test.—Another interesting line of argument regarding oxidising or reducing state of the substratum has been suggested by routine tests for the presence of nitrates in the soils examined. The test employed is as follows. Fresh soil is shaken with three times its volume of water, and after one hour seven drops of 0·02% diphenylamine in concentrated sulphuric acid are added to one drop of the clear extract. The depth of the blue colour produced is noted, and four degrees of intensity being used for recording. Table VIII gives the results of this test for a few representative samples. It will be noted that almost all the soils give positive results with the above test, showing thereby the absence of reducing conditions. The

alga grows under highly oxidising state of the substratum, a conclusion which has also been arrived at by the study of the oxidation-reduction potential and the ammonium-thiocyanate results.

TABLE VIII

Showing results of diphenylamine test

No. of Sample	Nitrate
1	++
7	++
32	+
39	+++
46	Slight
82	++
85	+
88	++++
94	+++

Discussion

Scytonema ocellatum Lyngb. forma *minor* Bharadwaja grows all the year round, *i.e.*, in rainy, winter and summer seasons, and hence it is considered to be a perennial form. Its growth in various seasons is conditioned by the amount of moisture in the soil. It has been observed from the experimental results, as well as from the direct study of the soil samples freshly collected from the fields, that even at a soil moisture of 10% of the water-holding capacity the alga grows very well. It is therefore inferred that the moisture requirements of a soil alga as the one considered here, is considerably less (*cf.* Fritsch, 1922 and 1936). It is also seen that the dried up papery incrustations of the plant regain growth after two days on the addition of water. The plentiful growth continues upto 40% soil moisture of the water-holding capacity, beyond which the number of individuals of the alga goes on decreasing. This indifference of the plant to increasing moisture levels in the soil is explained by the fact that under such conditions the oxidation-reduction potential decreases, *i.e.*, there is an inverse proportionality between the two, a view also maintained by Pearsall (1938). Under such low E_7 values (359-376 m.V.) the growth is impeded to a great extent. A study of the redox potential at lower percentages of soil moisture shows that though the E_7 values are sufficiently high (470-495 m.V.) the plant is not able to grow due to the limiting moisture conditions. The general conclusion that may be derived from the redox potentials of the soils is that the E_7 range for the alga in question varies between 410 and 456 m.V. It is noted at the same time that the number of individuals increases with the increase in E_7 values.

On the whole, it is maintained that the oxidising state of the substratum helps a good deal in the growth of the alga. A confirmation of the same behaviour is also given by the ammonium-thiocyanate and diphenylamine tests, which respectively show that there is total absence of reduction, and hence the latter test is positive. The pH range of the alga, as has been mentioned in the previous pages, varies between pH 6·4 and pH 7·4. The number of the plant is more at higher pH values provided the oxidation-reduction potential of the soil is high. It has been seen that the alga shows an indifference to slight changes in the pH of the substratum. In contrast to the unimodal curves of Salisbury and others and the bimodal curves of Pearsall (1926) and Volk (1931), as quoted by Braun-Blanquet (1932), there is found to be a many-peaked curve. There is no fixed optimum and it varies both on the acid and alkaline side of the pH scale.

It is thus seen that the above studies imply the conception of limiting factor of some kind. As in most of the other branches of biology, it has become increasingly clear that it is impossible to consider the effect of one isolated factor alone (Raunkiaer, 1934). We are always dealing with a complicated combination of factors and that the relative values of these may differ immensely in new combination.

The indifference of the plant to changes in the reaction of the substratum and organic content emphasises its role as a colonist, which is further proved by the study of the redox potential and low moisture requirements of the alga. It shows therefore that *Scytonema ocellatum* Lyngb. forma *minor* Bharadwaja grows well on soils which behave adversely with respect to organic matter, moisture and plant nutrients, which characterise a new ground. It may therefore be concluded that primitive plants of this nature are likely to play an important role in the enrichment of the soil for the subsequent growth of higher plants. Such autecological studies on other genera and species of the Myxophyceæ are likely to bring forth further fruitful results in this direction.

Summary

The autecology of *Scytonema ocellatum* Lyngb. forma *minor* Bharadwaja has been studied, especially with respect to its soil requirements.

The alga has been found to grow throughout the year and hence it is considered to be a perennial form.

The seasonal distribution of the plant is governed by the necessary moisture in the soil. A prolific growth takes place at a moisture of 20–40% of the water-holding capacity of the soil.

The growth of the alga is not affected by differences in the organic content of the substratum; but, on the other hand, it is the balance between carbon and nitrogen, *i.e.*, C/N ratio of the soil which is the controlling factor.

The pH range varies between 6·4 and 7·4, but maximum growth results at higher pH values provided the redox potential is fairly high.

The oxidation-reduction potential varies between 410 and 456 m.V. (E_7 values), and at higher values there is an increase in growth.

The ammonium-thiocyanate and diphenylamine tests show that the soil samples characterising the growth of the alga indicate intense oxidation intensity.

It is concluded that, on the whole, the plant enjoys an oxidising nature of the substratum.

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y. Bharadwaja for the kind guidance and criticism throughout the course of this investigation.

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SOME METACERCARIAL FORMS OF *CLINOSTOMATIDAE* (TREMATODA) FROM INDIA

By G. D. Bhalerao, D.Sc., Ph.D., F.Z.S., F.R.M.S., F.A.Sc.

[*Research Officer (Helminthology), Imperial Veterinary Research Institute, Izatnagar*]

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Euclinostomum indicum n. sp.

A few specimens were obtained from the cœlomic cavity of *Ophiocephalus punctatus* in Poona. The worms are thick and fleshy with both extremities bluntly rounded. The anterior end and the sides of the body are bent ventrally. They measure 4·5 mm. in length and 1·72 mm. in maximum breadth. The oral sucker is terminal and measures 0·22 mm. in diameter. The prepharynx is small, being only 0·085 mm. long. The pharynx measures 0·043 × 0·062 mm. The œsophagus is very short and can be seen only in sections. The intestinal cæca pass up to the posterior end of the body. Each cæcum has eight branches externally, of which the fifth divides into two small sub-branches distally. The ventral sucker is situated in the second fifth of the body and measures 0·75 mm. in diameter. The excretory pore is situated subterminally at the posterior end of the body on the dorsal side. The excretory bladder is small and gives out two lateral branches which ramify profusely in the body.

The gonads are situated in the fourth fifths of the body and the testes are tandem. The anterior testis is more or less W-shaped and measures 0·18 × 0·6 mm. The posterior testis is Y-shaped and measures 0·265 × 0·5 mm. The cirrus sac lies horizontally in front of the anterior testis and contains a coiled vesicula seminalis, the pars prostatica and a small ductus ejaculatorius. The pars prostatica is surrounded by the prostatic cells. The genital pore is situated 0·10 mm. behind the acetabulum.

The ovary is almost round, measures 0·165 × 0·135 mm. and is situated in the inter-testicular area, slightly to the right of the middle line. A very thin, sinuous oviduct emerges from the ovary and after a short distance dilates into the oötype which is surrounded by the cells of the shell gland, the latter measuring 0·1 × 0·055 mm. A somewhat thicker oviduct coils in the inter-testicular area and continues as the utero-duct which curves on the left side of the anterior testis and continues to meet the uterus at its anterior end. The uterine sac is 0·75 mm. long and terminates 0·3 mm. behind the ventral sucker, at about the anterior two-fifths of the body.

Up to the present time, only three species of *Euclinostomum* have been recorded, *viz.*, *E. heterostomum* (Rudolphi, 1809), *E. clarias* (Dubois 1929) and *E. multicæcum* (Tubangui and Masilungan, 1935). From these species, the present form can be distinguished by the anterior extent of the uterine sac which terminates at about the anterior tow-fifths of the body. Moreover in the last two species the cirrus sac has not been mentioned. The gonads in the present species lie in the fourth fifths of the body. In the location of the cirrus sac, it resembles *E. heterostomum* but, as has been already mentiond, it differs from it in the anterior extent of the uterine sac and the utero-duct. The ratio of the oral to the ventral sucker in *E. heterostomum* is 1 : 4·5, whereas in the present form it is 1 : 3. From this it is clear that the species in question is a new one, for which the name *Euclinostomum indicum* is proposed.

Clinostomum prashadi n. sp.

Numerous specimens obtained from an unidentified fish and deposited in the collection of the Central Research Institute, Kasauli, were sent to the writer for identification.

The worms are elongate and thin, with the anterior end and the sides bent ventrally. They measure 3·6–4·73 mm. in length and 1·3–1·77 mm. in maximum breadth which is attained in the region of the gonads. The mouth is subterminal and is surrounded by the oral sucker measuring 0·17–0·19 × 0·18–0·215 mm. The prepharynx is 0·028 mm. long and the pharynx measures 0·4 mm. in diameter. The intestinal cæca are simple and terminate near the posterior end of the body. The ventral sucker is situated at about the anterior fourth of the body and measures 0·65–0·68 × 0·52–0·56 mm., being about three times larger than the oral sucker. The excretory pore is situated at the posterior end of the body.

The gonads are situated in the third quarter of the body. The anterior testis is somewhat irregularly lobed and it measures 0·335 × 0·35 mm. The posterior testis is Y-shaped and measures 0·245 × 0·29 mm. The cirrus sac measures 0·41 × 0·09 mm. and is situated on the right side of the anterior testis. It contains the vesicula seminalis, the pars prostatica and the ductus ejaculatorius. The pars prostatica is surrounded by the prostatic cells. The genital pore is situated at the anterior border of the anterior testis.

The ovary is irregularly lobed, measures 0·085 × 0·075 mm. and lies posterior to the cirrus sac. The uterine sac terminates immediately behind the ventral sucker. The utero-duct joins the uterine sac close in front of the anterior testis. The metraterm is 0·135 mm. long. The vitellaria extend from the posterior border of the ventral sucker to the ends of the intestinal cæca.

Among the metacercarial forms recorded so far, the present species resembles *Clinostomum piscidium* Southwell and Prashad, 1918. It, however, differs from *C. piscidium* in the position of the gonads in the body, in the extent of the vitellaria and in the ratio of the oral to the ventral sucker. Moreover, the present form is thin and very much elongated in shape. It is, therefore, proposed to regard it as a new species for which the name *Clinostomum prashadi* is proposed in honour of Dr. Baini Prashad.

Clinostomum dasi n. sp.

Only one specimen obtained from *Saccobranchus fossilis* in Hyderabad (Deccan) was available for study. The worm is flat and is constricted at the level of the middle of the ventral sucker. It measures 3·54 mm. in length and 1·33 mm. in maximum breadth which is attained immediately in front of the anterior testis. The mouth is subterminal and is surrounded by the oral sucker measuring 0·23 × 0·103 mm. The ventral sucker is 0·52 mm. in diameter and is situated at about the anterior fourth of the body. The pharynx measures 0·058 × 0·041 mm. The intestinal cæca have a crenated outline and terminate near the posterior end of the body. The excretory pore is subterminal.

The gonads lie in the middle thrid of the body. The anterior testis is irregularly lobed and measures 0·2 × 0·25 mm. The posterior testis is trilobed and measures 0·22 × 0·36 mm. The cirrus sac, measuring 0·44 × 0·17 mm., is situated in the inter-testicular zone. It contains the vesicula seminalis, the pars prostatica and the ductus ejaculatorius. The genital pore is situated on a level with the posterior border of the anterior testis.

The ovary is irregularly lobed, measures 0·065 × 0·077 mm. and is situated immediately in front of the anterior testis, slightly to the right of the middle line. The uterine sac is 0·58 mm. long and terminates 0·13 mm. behind the posterior border of the ventral sucker. The utero-duct unites with the uterine sac immediately in front of the anterior testis. The vitellaria were not visible. The metraterm is 0·03 mm. long.

This form is allied to *C. chrysichthys* from which it differs in the position of the gonads and in the point of union of the utero-duct with the uterine sac. It is, therefore, proposed to regard it a new species for which the name *Clinostomum dasi* is proposed in honour of Professor B. K. Das.

Clinostomum gideoni n. sp.

Two specimens obtained from the gills of *Barbus sephre* were available for study. The site of infection is noteworthy.

The worms are elongate and moderately fleshy. Their anterior ends are bent ventrally. They measure 5·43—6·62 mm. in length and 1·67–1·75 mm. in maximum breadth which is attained in the testicular zone. The mouth is subterminal and is surrounded by the oral sucker, the latter measuring 0·33–0·36 × 0·32–0·43 mm. The pharynx measure 0·075–0·1 mm. in diameter. The internal cæca have a crenated outline and terminate slightly in front of the posterior end of the body. The excretory pore is subterminal.

The gonads lie in the second third of the body and the genital pore is situated along side of the middle of the anterior testis. The anterior testis is irregularly lobed and measures 0·45–0·68 × 0·36–0·65 mm. The posterior testis is lobed, somewhat Y-shaped and measures 0·23–0·48 × 0·47–0·67 mm. The cirrus sac lies on the right side of the anterior testis and measures 0·4–0·46 × 0·185–0·225 mm. It contains the vesicula seminalis, the pars prostatica and the ductus ejaculatorius.

The ovary is irregularly lobed, measures 0·15–0·23 × 0·12–0·21 mm. and lies immediately behind the cirrus sac. The uterine sac is 0·19 mm. long and terminates 0·107 mm. behind the ventral sucker. The utero-duct joins the uterine sac at about its posterior two-fifths. The vitellaria extend from the posterior border of the ventral sucker or slightly in front of it to a distance of 0·62 mm. from the posterior end of the body.

The present form somewhat resembles *C. chrysichthys* but differs from it in the point at which the utero-duct joins the uterine sac, in the nature of the testes and above all in the position of the gentital pore which is situated on a level with the middle of the anterior testis. It is, therefore, proposed to regard it a new species for which the name *Clinostomum gideoni* is proposed in honour of Prof. Gideon.

Clinostomum piscidium Southwell & Prashad, 1918

A few specimens obtained from the body cavity of *Nandus nandus* from Poona were available for study. These on examination proved to be *Clinostomum piscidium* (Southwell and Prashad, 1918). A few observations made on a single mounted specimen are recorded below:

The body is thick, fleshy and measures 3·5 mm. long and 1·96 mm. in maximum breadth which is attained in the testicular region. The oral sucker measures 0·18 mm. in diameter. The acetabulum measures 0·6 × 0·48 mm. The gonads are situated immediately behind the middle of the body. The cirrus sac is situated on the right side of the anterior testis, is slightly curved and measures 0·35 mm. in length. The genital pore is

situated on a level with the anterior margin of the anterior testis. The uterus extends anteriorly almost as far as the posterior border of the acetabulum. The utero-duct joins the uterus slightly in front of the anterior testis. In the original description the pharynx is said to have been absent but in the specimen under examination a small pharynx was found to be present. The tendency for the retraction of the anterior end of the body bearing the oral sucker is usual. There is also a tendency for both the ends of the body and the sides to retrovert ventrally.

Dorsal view of *Euclinostomum indicum* n. sp.

Acknowledgment

For the forms dealt with in this paper, the writer is indebted to Prof. J. N. Karve of Poona, Professor Gideon of Dharwar, Professor B. K. Das of Osmania University and Dr. V. T. Korke, formerly of the Central Research Institute, Kasauli.

A STUDY OF THE GIANT STAR GRASS (CYNODON SP.) FOR HCN CONTENT, YIELD AND PALATABILITY

By L. S. S. Kumar and W. V. Joshi

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Introduction

Pole Evans first reported on the Giant Star grass in his note "A Grass Leviathan from East Africa" in *Nature*, July 1, 1939. Giant Star grass belongs to the genus Cynodon and is a species allied to *C. dactylon* (*hariali* or *doob*). This grass came to be introduced into cultivation at the grass plots laid down at Kabete, near Nairobi, Kenya, by the chance introduction of its seed from sod of a fine-leaved lawn grass from Uganda. The performance of this grass from the very first was so remarkable and arresting that expeditions sent by the South African Government into Central and East Africa on collection of useful pasture grasses were instructed to be on the look-out for this grass. The expedition has since reported on the natural occurrence of this grass in some parts of Kenya and Uganda.

Trials taken on this grass at the Department of Agriculture's Grass Introduction Research Station at Pretoria have shown that it is highly useful for hay, silage and erosion control. Pole Evans in his note reports that it is customary, among stock owners who have grazed their animals in the country around Nakuru and which have developed "Nakuritis," presumably a deficiency disease, to send them to grass areas round Lake Solai which are rich in Giant Star grass for recovery from this disease.

The photograph included in the note referred to above, illustrates clearly the remarkable habit of this Star grass. A single runner in five and a half months attained a length of 48½ feet besides giving rise to numerous side shoots all along its length; within the same period a single plant covered 8,000 square feet of ground. The plant is remarkable in that it anchors itself to the ground firmly by rooting itself at every node, thus proving of immense value as an excellent soil binder. In view of these excellent characteristics, it was proposed to introduce this grass into Bombay Province.

Material and Method

The first sample of seed of Giant Star grass received from Mr. D. C. Edwards of the Department of Agriculture, Kenya, totally failed to germinate. The same officer was kind enough to supply living material and sample of seeds of the Star grass a second time by airmail. In all, four rooted cuttings were received which were immediately planted in 12″

pots containing good river earth. The rooted cuttings established themselves very quickly. The second sample of seeds received germinated fairly satisfactorily and a number of seedlings was obtained. These were later transplanted into the ground where they grew very rapidly. The grass showed all the characteristics of growth reported by Pole Evans.

Soon after the Giant Star grass was established at Poona, which receives an annual rainfall of 22", and where the months of March, April and May are very warm with the temperature varying between 100° F. to 109° F. the writers came across a note by the Chief Chemist to New South Wales Government, in the *Agricultural Gazette*, 1940, Vol. 51, No. 4, in which a warning had been given against the possible danger of stock-poisoning from feeding on Giant Star grass as its HCN content was high as to be lethal. A similar warning was received from the Chief of the Division of Soil and Veld Conservation, South Africa, from whom seed sample of the Star grass had been received, but which failed to germinate. In view of these warnings, any intention of distributing the grass for introduction in various parts of the Bombay Province was given up for the time being and instead, trials were conducted to test the stock-poisoning effect, HCN content, yield, etc., of the Giant Star grass.

TABLE I

Prussic acid content of Giant Star grass grown in the Economic Botanist's area, Poona, from rooted cuttings received from Africa, on 28th January 1940*

Condition of crop at the time of sampling	Date of analysis	Hydrocynic acid content	
		Local *Hariali*	Giant Star
1. Raised from rooted cuttings. Crop not yet in flower	17–6–40	0·039	0·043
2. 1st ratoon crop from No. 1 above ..	30–9–40	0·004	0·008
3. The above crop soon after commencement of flowering	28–11–40	0·002	0·006
4. 2nd ratoon crop, shoot with plenty of young leaves. The plot was previously harvested on 11–12–40, the shoots were only 5 weeks old when sent for analysis	20 1–41	0·001	0·057
5. The above crop sent to analysis when 2½ feet tall. Crop not in flower. Leaf and stem samples analysed separately	26–2–41	Stems 0·0009 Leaves 0·002	Stems 0·019 Leaves 0·067
6. A sample of Giant Star grass raised from seeds. Crop in flower ..	24–4–41	0·003	0·013
7. Crop, 1 to 1½ feet tall	2–6–41	..	0·04

* The analysis for HCN and other content was done by the Agricultural Chemist to Government, B. P., Poona. The materials sent for analysis were from different plantings and different ratoons.

In Table I is given the HCN content of the Giant Star grass together with that of *hariali* (*doob* grass–*Cynodon dactylon*). It is observed that the HCN content of local *hariali* (*Cynodon dactylon*) is in every instance less than that of the Giant Star grass. Item (5) shows that the leaves of the Star grass contain considerably more HCN than its stem. This agrees with the observations of Acharya (1933) and Franzke *et al.* (1939) reported for *Sorghum*.

In order to determine the relation of age to the HCN content of the grass, a large number of cuttings was simultaneously planted on a day and samples from different cuttings were cut periodically and were analysed. Table II gives the results of such analyses.

TABLE II

Hydorcynic acid content of Giant Star grass raised from cuttings, planted on 15th July 1941

(Samples for analysis were obtained only once from each cutting)

Date of cutting specimens for analysis	Age of samples from date of planting in days	Moisture content %	Prussic acid content %	Remarks
13—8–41	29	78·00	0·049	There was no irrigation or rain after planting till 13/14–9–41
27—8–41	43	74·00	0·048	
9—9–41	56	33·00	0·03	
20—9–41	67	68·00	0·041	Hand watering on 9–9–41 after the sample was cut on 9·9·41. Heavy rain on 13/14–9–41.
1–10–41	78	69·00	0·053	
16–10–41	93	67·00	0·044	
28–10–41	105	63·00	0·029	
28–11–41	135	51·00	0·0093	
18–12–41	156	51·00	0·003	
9—1–42	178	50·00	0·0057	
30—1–42	199	49·00	0·005	

The above table shows that with the advance in age of the plants raised from cuttings the HCN content does not show any steady decrease as observed in *Sorghum* by Acharya (1933) and Franzke *et al.* (1939). On the other hand the HCN content of the Giant Star grass somewhat fluctuates in amount showing no exact correlation with the age of the plant. Table II however shows what appears to be some correlation between the moisture content of the grass and its HCN content. High moisuture content is associated with high HCN content although this agreement is not quite perfect. Acharya points out that *Sorghum* plants growing under drought

condition had greater HCN content than those receiving sufficient water for their growth. So far as the Giant Star grass is concerned this observation does not appear to hold good.

Feeding and Palatability Trial

Acharya quoting Couch states that 0·02 per cent. and above of potential prussic acid may cause death of cattle and horses if as little as 5 lbs. is eaten. Green material of the Giant Star grass was fed to different types of animals to test for its stock-poisoning effect, if any. The results of the feeding trials are given in Table III.

TABLE III

Summary of feeding trials of Giant Star grass

Kind of Animal	Dates of feeding	Quantity of Giant Star grass consumed
1. Adult bull weighing over 1000 lbs. ..	12–9–40 to 14–9–40	48 lbs. in 48 hours
2. Adult female buffalo weighing over 1000 lbs.	10–12–40 to 11–12–40	34 lbs. in nearly 31 hours
3. Young male buffalo calf aged 6 months and 2 days weighing 258 lbs.	15–4–41 to 26–4–41	78½ lbs. in 11 days and 9 hours
4. Young male buffalo calf aged 7 months weighing 274 lbs.	25–5–41 to 3–6–41	114½ lbs. in 9 days and 6½ hours
5. Three adult farm bullocks	26–4–41 to 28–4–41	285 lbs. in 2 days and 7 hours
6. Adult farm animals (3 or 4 bullocks) ..	17–6–41	Actual quantity sent 118 lbs. Actual quantity consumed 81 lbs.
7. Pony weighing 280 lbs.	10–11–41 to 15–11–41	52 lbs. for 6 days
	22–11–41 to 24–11–41	26 lbs. for 3 days
8. Sheep	One week	3 to 4 lbs. per sheep per day

During the above feeding trials the prussic acid content of local *hariali* varied between 0·001% to 0·039% and that of the Giant Star grass from 0·006% to 0·057%. In all four different types of animals both young and adults were tried. The feeding trial lasted from one to as many as eleven and half days. The quantity of the Star Grass fed during the period varied from a few lbs. per animal to 114 lbs. None of the animals showed any ill-effect at any time during the trials.

Of the animals to which the Giant Star grass was fed none relished the grass. The animals had to be forced to feed on the grass by starving them of all other feeds. Thus they were obliged to eat the grass. Sometimes when the grass was mixed with other fodder, the animals would pick out the fodder to which they were accustomed and leave out the Star grass. Our experience shows that the Star grass is not relished by animals and that they avoid it if other fodders are available.

Yield Trial

To estimate the yield of the Giant Star grass it was compared with that of Guinea grass (*Panicum maximum*). Table IV gives the yield of the two grasses.

TABLE IV

Comparison of yields of Guinea and Giant Star Grass from 27–2–41. *Six equal sized plots of each. Both plots sown on* 2–12–40 *and replicated according to ABBA plan*

Dates of cuttings	Guinea Grass		Giant Star Grass	
	lbs.	ozs.	lbs.	ozs.
26—4–41	166	5	201	..
11/16—6–41	262	2	..	..
17—6–41	..	..	199	8
15/17—7–41	260	10	..	..
24—7–41	..	..	84	..
23/25—8–41	138	7	..	..
3—9–41	..	..	57	1
27/29—9–41	149	4	..	..
8–10–41	..	..	23	..
31–10–41 } 2–11–41 }	109	..	..	..
26–11–41	..	..	33	2
19/20–12–41	87	8	..	..
2—2–42	..	..	38	8
17/19—2–41	56	14	..	..
11/13—4–42	153	2	..	..
14—4–42	..	..	97	1
19—5–42	..	..	50	7
Total of 9 harvests	1,383	4	783	11

Except for one occasion the two grasses were not cut on the same day as the rate of growth of each differed considerably. Although both the grasses were planted on the same day, Guinea grass was far in advance of the growth of the Giant Star grass. Both the grasses were periodically irrigated. Under irrigated condition it is seen that Guinea grass out-yields the Giant Star grass by 76·5%. The comparative yield of the same two grasses under dry condition is yet to be ascertained.

A comparison of the proportion of stem to leaf of *hariali* (*Cynodon dactylon*) and the Giant Star grass showed that the latter contained a greater proportion of stem which on account of being very fibrous and tough are not eaten by the cattle.

TABLE V

Proportion of stem to leaf of Cynodon dactylon *and of Giant Star grass*

Name of the grass	Proportion of fresh			
	Leaves		Stems	
	ozs.	%	ozs.	%
Giant Star	13	44·8	16	55·2
Local *hariali* (*Cynodon dactylon*) ..	12	48·0	13	52·0

The rate of growth of the Giant Star grass was compared with that of *hariali*. A germinated seedling and a sprout from a cutting of the Star grass were planted on the same day with a sprouted cutting of the *hariali*. The longest runner of the Star grass from the cutting measured 42 feet after 22 months' growth, while that from the seedling measured 57 feet. In this period two runners of the *hariali* had each grown 9 feet in length only. Although the growth of the Star grass is not as rapid as that reported by Pole Evans for conditions under Pretoria, yet the Star grass is decidedly a very rapid grower compared to *hariali*.

TABLE VI

Rate of growth of Star Grass and Hariali

Name of the grass	Date of planting	Date of measuring	Longest runner in ft.
Star Grass—			
(*a*) seedling	30–7–40	10–6–42	57
(*b*) cutting	do.	do.	42
Hariali—			
cutting	do.	do.	9

Discussion

The Giant Star grass, a species of Cynodon, is considered to be of great importance in its home in Kenya and Uganda. It is an extremely rapid grower, produces luscious green fodder which has the property of reconditioning and effecting recovery in animals suffering from " Nakuritis, " from which disease cattle suffer in areas round Nakuru (Kenya). Its habit

of covering very extensive areas in a short period, profusely branching and rooting at every node makes it of importance as a very useful soil binder. Trials carried out in the Veld in South Africa have shown that the grass is higly susceptible to frost injury and appears to be more suitable to warm climates. Tests carried out at Poona, in the Bombay Deccan, has shown that the grass is a quick grower and in spite of the higher degree of prussic acid content than that of *hariali* (*C. dactylon*) it has not caused any stock-poisoning in cattle, horse or sheep. The drawback of this grass appears to be mainly its unpalatability. Animals fed on this grass do not relish it unless forcibly fed. Another defect appears to be that the grass completely dies down during the hot months when the temperature ranges between 100° F. to 108° F. Therefore its drought-resistant characteristics are doubtful. It is a poor yielder compared to Guinea grass which is much relished by cattle.

Summary

(1) The Giant Star grass grows very rapidly and has a good soil binding effect.

(2) The HCN content of the Giant Star grass varies from 0·003 to 0·053 per cent. while that of *hariali* (*Cynodon dactylon*) varies from 0·001 to 0·039 per cent.

(3) Feeding trials carried out on bullock, buffalo, pony and sheep showed no stock-poisoning effect.

(4) Animals fed on Giant Star grass have not relished the grass.

(5) Under irrigated conditions Guinea grass (*Panicum maximum*) has yielded 76·5 per cent. more than Giant Star grass.

(6) The proportion of stem is greater than leaf in the Giant Star grass as compared to *hariali* (*C. dactylon*) in which the proportion is very nearly equal.

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Giant Star (*Cynodon* sp.) grass grown to a height of three and half feet

LIFE-HISTORY AND BIONOMICS OF *PSEUDOCOCCUS SACCHARICOLA* TAKH. (HOMOP-COCCIDÆ)—A NEW PEST OF SUGARCANE IN INDIA

By S. Ram Mohan Rao, D.Sc.

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Introduction

Damage caused by the mealy bugs in Louisiana has been estimated at one time to be about five tons per acre. The infestation of *Pseudococcus sacchari* in Egypt (Hall, 1922) was so intense that the industry was virtually threatened with extinction. Recently Dymond (1929) has shown that the mealy bug " is to a definite extent responsible for the production of dextro-rotatory gum with colloidal matter of a practically objectionable nature ". The damage done by the three mealy bugs, *Dactylopious* (*Trionymous*) *sacchari*, *Ripersia sacchari* and *Aclerda japonica* which are often found as dense colonies inside the sheathing leaves has been expressed by Lefroy (1908) in the following words : " Actual lessening of the yield of sugar is stated to occur as well as deterioration in quality and we may rank the three species collectively as being of considerable degree of importance. "

Another reason why the presence of the mealy bugs is not to be looked upon with equanimity is their probable role in the transmission of virus

diseases. Smyth (1919) and Bruner (1922) have definitely incriminated the mealy bug as a vector of sugarcane mosaic.

On account of their economic importance *Pseudococcus sacchari* (*Trionymous sacchari*) the pink mealy bug of sugarcane was the subject of an excellent memoir by Uichanco (1932). Swezey (1913) also studied the biology of the three sugarcane mealy bugs and their natural enemies in Philippines.

Our knowledge of the bionomics of the sugarcane coccids in India is very meagre. In the following pages an attempt is made to study the life-history and biology of *Pseudococcus saccharicola* which was found in considerable numbers at the Imperial Sugarcane Station, Coimbatore, where the author had the opportunity of first encountering the pest. Its occurrence in other places in India has not so far been reported. It is hoped that the information obtained herein will enable us to tackle the problem of controlling the pest if and when it succeeds in entering other parts of India.

My grateful thanks are due to Dr. G. S. Thapar of the Lucknow University, Lucknow, for his help in the preparation of this manuscript for the press.

Material and Methods

The material for this study was collected from the Imperial Sugarcane Station, Coimbatore. For studying the habits of the larvæ and observing the number of moults passed through by each sex, freshly emerged first stage nymphs were removed from the mother and placed on pieces of sugarcane leaf in petri dishes. Enough moisture was provided to prevent the drying up of the leaves.

Geographical Distribution and Food Plants

Formosa is at present the only island infested with *P. saccharicola.* Its presence in Coimbatore was first detected by the author in 1939 and it seems to be the first record of this species from India. Excepting sugarcane, no other alternative host has been mentioned by Takahashi (1928) from Formosa. In India, however, some grasses (unidentified) besides sugarcane serve as alternative host plants.

Reproduction

Notwithstanding the fact that a large number of males are available for inseminating the females, reproduction seems to proceed by amphigony and parthenogenesis. That the females are really parthenogenetic is proved by the fact that the latter, after the last nymphal moult, oviposited in the normal manner even when no males were available to them.

Oviposition

Eggs are laid by the gravid females beneath their abdomen. The pre-oviposition period of the female is twelve days. Period of parturition is ten to twelve days. The number of eggs laid by each female varies from 200 to 300 with an average of 215. The female eventually dies after the last egg is laid.

Egg Stage

The eggs are creamy yellow in colour with a covering of mealy powder over them and measure 0·4 mm. in length and 0·19 mm. in breadth. The incubation period of the egg is very short. Just before eclosion, the egg is dorso-ventrally compressed and the fully developed embryo shows itself through the chorion. When ready to break through it the embryo performs movements inside the covering which pass from behind forward and which tend to widen the chorionic covering to the bursting point. Finally, the shell breaks at the anterior end and the young larva wriggles itself out of it. The whole process of emergence lasts for twenty to thirty minutes.

First Stage Nymph

It is cream yellow in colour and 0·75 mm. in length and 0·34 mm. in breadth. Immediately on emerging from the eggs, the nymphs remain quiescent for a day or two and then set out in search of suitable places for feeding. They, like the adult, are gregarious in habit. The antennæ are hairy and six-jointed. Legs are large and ungainly. Eight segments can be made out in the abdomen. The occellanæ lie on the margin behind the antennæ. The sex of the nymphs cannot be distinguished at this stage.

The Male Line

Second stage nymph.—The cream yellow colour of the first stage larva changes to light pink soon after the first moult. The larva, at the time of the formation of the cocoon, measures 1 mm. only. For sometimes, larvæ of both sexes feed together, but a few days before the second moult, the male-producing larva spins a cocoon. It is at this stage that the segregation of the two sexes takes place. All the male larvæ descend down the trunk of the young plants and pupate in the leafsheaths, while the female-producing ones remain feeding on the leaves.

The mealy powder which is actively secreted at this stage covers the body of the insect entirely. It is said that in some genera of the *Eriococcinæ*, the females lose their rostralis at the time of the first moult, but in *Pseudococcus saccharicola* the second stage larvæ not only retain the rostrum but

actively feed by inserting the stylets into the tissues of the host plant. This stadium lasts for six or seven days after which the next stage is attained.

The Prepupa

The prepupa is completely different from the second stage larva and is characterised by the complete absence of the rostrum, the imperfect division of the head, thorax and abdomen, the appearance of the wing buds and the immobility of the antennæ. The eyes are represented by a pair of occellanæ situated at the margin of the body just behind the antennæ. A slight conical projection from the last caudal segment representing the future stylus is also present. The anterior pair of legs are seen moving during the whole process of cocoon formation. In this connection, it may be mentioned that Newstead (1902) observed a similar thing in the case of the pupa of *Dactylopius sp.* (*Citri*). When denuded of the secretionary matter, the prepupa appears pinkish in colour. The prepupal stage escaped the notice of the earlier observers. The pupal stage is attained after a moult. The duration of the prepupal stage is two days only.

The Pupa

The pupa is distinguished from the prepupa by the presence of the wing pads, elongated antennæ, three pairs of eyes—a large pair situated dorsally and another pair ventrally and a small pair of occellanæ on either side and a well-developed stylus.

The head, thorax and abdomen are clearly delimited. The whole body is covered with a powdery secretion. If the cocoon is destroyed, it begins to construct a new one. The pupa moults once again to attain the adult form.

Adult Male

The adult male is a delicate and small creature with a pair of wings, hairy antennæ and well-developed stylus. Abdomen tapers posteriorly and a pair of slender waxy filaments are borne by the last caudal segment. Six eyes are present on its head and the colour of the body is reddish pink. Soon after its emergence from the cocoon, the male is sluggish but sets out in the quest of the female after a brief pause. The male is short lived, its average duration of life not exceeding three days. The number of segments in the antennæ is nine. The male of *P. saccharicola* moults four times and passes through five stages in its life-history. Lefroy (1908) observed only four stages in the case of *P. saccharifoli*, a species allied to *P. sachari-cola.*

The Female Line

Second stage female.—General colour of the body is light pink and it measures 0·75 mm. in length and 0·36 mm. in breadth. The antennæ are six-jointed. The last segment of the antenna is rather long and bears a number of long hairs. Even in the second instar, the male-producing larvæ cannot be distinguished from female-producing ones until the former begins to spin the cocoon. The whole body is covered over by a mealy secretion. The second stage female-producing larvæ pass into the next stage at the end of six days.

Third stage larva.—Colour of the body remains the same as before. Size of the young adult is 1·8 mm. in length and 0·8 mm. in breadth. This stage is so much like the adult female that there is little to distinguish it from the latter except difference in size. Pencils of wax grow out from the margin of the body. Antennæ are seven-segmented. Secretion of wax and honey-dew is copious at this stage.

Adult Female

Average length of the adult female is 3·2 mm. and breadth 1·5 mm. It is yellowish-brown in colour with a number of waxy filaments growing out of the margin. The last pair of the marginal filaments situated at the posterior end of the abdomen is the longest of all of them. There are 16 pairs of cerores corresponding to the 16 pairs of marginal filaments of wax. The insect is densely covered with wax. They are very sluggish and seldom move away from the spot once selected for feeding. Like *P. saccharifoli*, it is a leaf feeder and is generally found in the distal half of the leaf. In some cases, good many of these bugs can be found (Pl. IX, Fig. 1) in the angle between the first open leaf and the spindle. The honey-dew is copiously secreted by the female and ants are attracted by it in numbers. The duration of life of the adult female including the pre- and post-gestational periods is 25 to 27 days. The number of moults occurring in female of the genus *Pseudococcus* is not yet correctly known. Berlese (1893) believed that the female moulted four times like the male, while Newstead (1902) thought them to be two. A careful study of the life-history of *Pseudococcus saccharicola* showed that the female moults three times and has four instars.

Nature of the Damage

Larvæ of *P. saccharicola* have a habit of feeding in company. Not infrequently, three or four hundred larvæ belonging to different stages are collected together in a square inch of leaf surface. Large quantity of sap are sucked up by them in consequence of which the host plant may be killed

if it happens to be young. A black sooty mould develops on the leaves upon which the honey-dew collects. Early symptoms of injury to the leaves are shown by the presence of yellow spots on them which eventually turn reddish-brown. A number of leaf tips become rolled up longitudinally and assume a reddish-brown colour.

Natural Enemies

Predators-Brumas suturalis Fabr., an unknown variety belonging to the family *Coccinellidæ*, has been found to feed on the nymphs. The destruction caused by this beetle to the mealy-bug population is negligible. Of the species of ants attending these bugs, the most important are *Camponotus compressus* and *Solenopsis geminata.*

Parasites.—Some unidentified chalcid parasites have been reared from the nymphs of *P. saccharicola.* The parasites are found in sufficient numbers and it is hoped to report on them at a future date.

Schedule of the Life-History Stages of the Female

Stage	Duration Days	No. of antennal joints	Colour	Remarks
1	6 to 8	Six	Creamy yellow	..
2	6 to 7	do.	Pale pink	..
3	6	Seven	Brownish pink	...
Adult	27	Eight	do.	Sixteen filaments round the margin

Schedule of the Life-History Stages of the Male

Stage	Duration Days	No. of antennal joints	Colour	Remarks
1	6 to 8	Six	Creamy yellow	..
2	6 to 7	do.	Pale pink	Begins to form the cocoon two days before the second moult
Prepupa	2	..	do.	..
Pupa	5	..	do.	...
Adult	3	Nine	Reddish pink	Copulation with female takes place

Summary

1. An account of the life-history and biology of *Pseudococcus saccharicola* Takh. is given in this paper.

2. Reproduction takes place both by amphigony and parthenogenesis.

Plates VIII and IX will appear in Vol. XVI, No. 4, Section B.

3. About 230 eggs are laid by the gravid female in 12 days. The incubation period of the eggs is very short.

4. The male has five stages in its life-history and moults four times. The female moults three times and there are four stages in its life-cycle. Distinguishing characters of the various stages are set forth in the text.

5. Some of the predators and parasites of *P. saccharicola* are mentioned.

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EXPLANATION OF THE PLATES

PLATE VIII

Fig. 1. Larvæ of second and third stages of the female clustered together on the leaf. Note the marginal tassels and profuse waxy secretion in these stages.

Fig. 2. Prepupa—showing the wing buds.

Fig. 3. Ventral view of the second stage male-producing larva showing secretion.

Fig. 4. Adult female showing marginal filaments.

Fig. 5. Pupa—showing well developed stylus, wing pads.

Fig. 6. Adult male.

PLATE IX

A fairly young sugarcane plant showing the infestation of *P. saccharicola*. *Componatus compressus*, the black ant is seen feeding upon the honey-dew.

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STUDIES ON THE TREMATODE PARASITES OF REPTILES

Part I. A New Trematode, *Acanthostomum indicum* belonging to the Family Acanthostomidæ, from the Intestine of a Crocodile

By Bipin Bihari Sinha

(Research Assistant, Helminthiasis Scheme, Lucknow University, Lucknow)

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Looss (1899) erected the genus, *Acanthostomum*, for the reception of the species *A. spiniceps* and *A. coronaris*. A year later (1900) the same author changed the generic name to *Acanthochasmus* on the ground of preoccupation of the name *Acanthostoma* by a genus of an insect. Subsequently, Braun (1899) added another species, *A. scyphocephalus*; while Odhner (1902) described two species, *A. productus* and *A. vicinis* respectively. Poche (1925) pointed out that since the name *Acanthostoma* and not *Acanthostomum* was preoccupied the change in name proposed by Looss was against the Rules of Zoological Nomenclature and therefore on grounds of priority the name *Acanthostomum* was retained. Bhalerao (1926) described the species *Acanthochasmus burminis* from the intestine of *Tropidonotus piscator* from Rangoon. Thapar and Ali (1929) have reported its presence from the same host at Lucknow. More recently, Bhalerao (1936) has discussed the nomenclature of the two generic names, and has agreed to Poche (1925) to give preference to the name *Acanthostomum*. The present paper deals with a new species of the genus recovered from the intestine of a crocodile from the river Tehri at Gonda in the United Provinces.

The present work was carried out in the Zoological Laboratories of the Lucknow University under the direction of Dr. G. S. Thapar and forms a portion of the author's D.Sc. thesis, which is now being published in the form of a series of papers on new and unrecorded forms only. The writer is deeply indebted to Dr. Thapar for the constant guidance and interest he has taken in this work. He made several helpful suggestions in the preparation of the manuscript for the press and also placed the use of his personal library at my disposal.

Acanthostomum indicum sp. nov.

The body is elongated, cylindrical and transparent, and measures 6·2–7·33 mm. in length and 0·36–0·45 mm. in greatest breadth which is

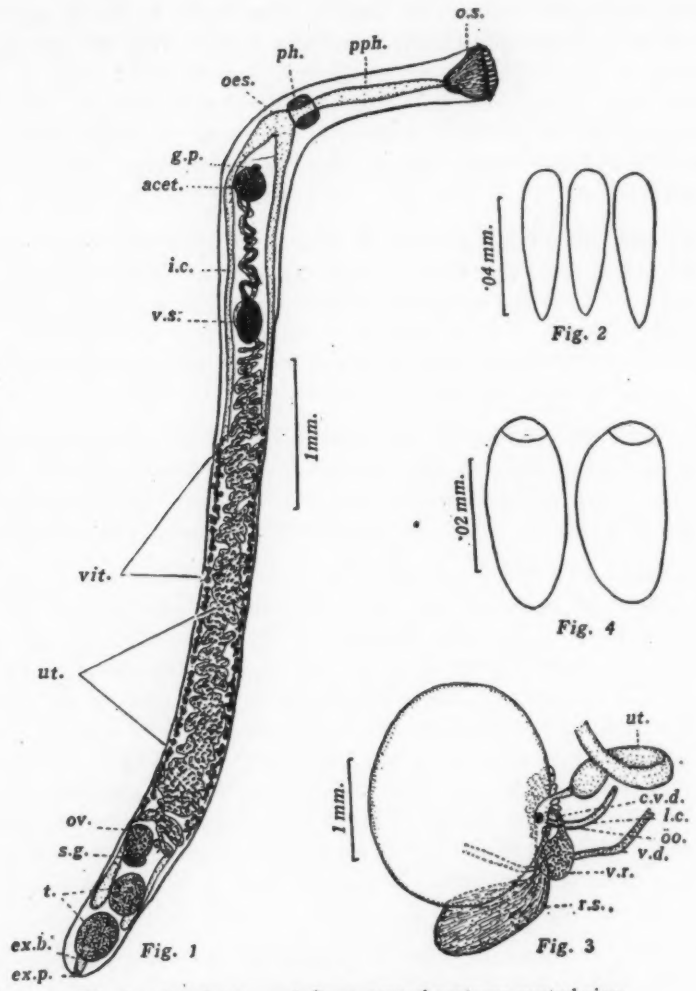

Fig. 1. *Acanthostomum indicum*, general anatomy, ventral view.
Fig. 2. Oral spines highly magnified.
Fig. 3. Ovary and öötype complex.
Fig. 4. Eggs highly magnified.

in the region of the ventral sucker. From this point the body gradually narrows down towards either end. In some specimens there is a slight bulging of the body wall in the region of the reproductive organs. The body is covered over by a thin cuticle armed with minute backwardly directed spines which are more numerous in the anterior region.

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The oral sucker is terminal and measures 0·35 by 0·32 mm. in size, being broader antero-posteriorly. It bears a single row of 22 large spines measuring 0·051–0·053 mm. in length and 0·0138–0·0144 mm. in greatest breadth. The ventral sucker is situated at 1·74 mm. from the anterior end, *i.e.*, at about $\frac{1}{4}$th of the body length. It is circular in shape and measures 0·2 mm. in diameter, being smaller than the oral sucker. The ratio of the two suckers is 5: 3.

The oral sucker is followed by a long prepharynx, 0·865 mm. long. The pharynx is large and more or less globular in shape, measuring 0·181 by 0·181 mm. The œsophagus is very short and immediately divides into the intestinal cæca. The point of bifurcation is a little in front of the ventral sucker. The cæca are almost of uniform width and run posteriorly almost to the end of the body.

The excretory pore lies at the posterior end of the body and leads into an elongated excretory bladder extending as far forwards as the anterior end of the ovary where it divides into two cornua. The lateral excretory ducts extend anteriorly along the sides of the body upto the pharynx.

The testes lie one behind the other, at the posterior end of the body, behind the ovary. Usually they are oval in shape, with the long axis lying antero-posteriorly. The anterior testis is slightly smaller than the posterior and measures 0·28 mm. by 0·25 mm. in size. The posterior testis lies a little behind the anterior testis and measures 0·35 mm. by 0·25 mm. in size. The vesicula seminalis is large, extending to 0·95 mm. behind the ventral sucker. It consists of a large posterior basal sac-like portion and an anterior narrow coiled portion. The sac-like portion measures 0·3 mm. by 0·13 mm. About the middle of the ventral sucker, the vesicula seminalis is continued into a small ductus ejaculatorius, that opens at the genital pore. A cirrus or cirrus sac is absent. The genital pore is situated immediately in front of the ventral sucker.

The ovary is ovoid in shape, 0·22 mm. by 0·174 mm., and lies to the right of the median line in front of the testes. The oviduct arises from the middle of the ovary and opens into the öotype situated slightly beneath the left margin of the ovary and surrounded by shell gland cells. Opening into öotype from the posterior side is the duct of the receptaculum seminis, which is situated behind the ovary. The Laurer's Canal also meets it at this point and itself opens to the exterior through a small pore on the dorsal surface of the body. The receptaculum seminis is large and oval in shape, measuring 0·127 mm. in length and 0·062 mm. in greatest breadth.

The vitellaria are poorly developed and consist of small follicles. They are situated on the lateral sides of the body, mostly covering the intestinal cæca and extending from 3·72 mm. behind the anterior end of the body to the level of the anterior end of the ovary. The ducts from the vitelline glands meet in the middle line in front of the receptaculum seminis and form a large vitelline reservoir from where the common vitelline duct takes its origin and joins the oviduct at the öotype.

The uterus arises from the öotype and passes anteriorly, describing small transverse coils between the ovary and the basal sac-like portion of the vesicula seminalis. Anteriorly it runs more or less parallel to the latter and opens at the genital pore. The uterus is full of small eggs with yellowish brown shells. The eggs are operculated and measure 0·032–0·034 mm. by 0·0145–0·016 mm. in size.

The present form differs from all the known species of the genus, except *A. burminis* in the general shape of the body, coiled nature of the vesicula seminalis, uterine coils and the vitellaria ending anterior to the ovary and in the presence of a large receptaculum seminis behind the ovary. Though it resembles *A. burminis* in all these characters, it can be easily distinguished from it, in the ratio of two suckers, in the prepharynx being very long, in the reproductive organs being confined to the extreme posteior end of the body, in the oral spines being 22 in number as against 24–27 in *A. burminis* and lastly in its being a crocodilian parasite. These differences are sufficient to justify the creation of a new species for the reception of these forms.

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LETTERING ON THE FIGURES

acet.	.. acetabulum.	*ov.*	.. ovary.
c.v.d.	.. common vitelline duct.	*ph.*	.. pharynx.
ex.b.	.. excretory bladder.	*p.ph.*	.. prepharynx.
ex.p.	.. excretory pore.	*r.s.*	.. receptaculum seminalis.
g.p.	.. genital pore.	*t.*	.. testis.
i.c.	.. intestinal cæcum.	*ut.*	.. uterus.
l.c.	.. Laurer's canal.	*v.d.*	.. vitelline duct.
æs.	.. œsophagus.	*vit.*	.. vitellaria.
oo.	.. öötype.	*v.r.*	.. vitelline reservoir.
o.s.	.. oral sucker.	*v.s.*	.. vesicula seminalis.

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A CONTRIBUTION TO THE MORPHOLOGY AND CYTOLOGY OF *MONOCHORIA HASTAEFOLIA* PRESL.

By I. Banerji and S. Haldar
(*Department of Botany, Calcutta University*)

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(Communicated by Dr. S. N. Das Gupta, ph.d., f.a.sc.)

Monochoria hastæfolia Presl. is one of the common marshy plants that is found all over the province of Bengal. According to Hooker (1875) it is distributed throughout India, Ceylon, Malay Islands, China and in the tropical and sub-tropical regions of the Old World.

The plant belongs to the family Pontederiaceæ to which the common Water-hyacinth (*Eichhornia crassipes*) also belongs. Both the plants are characterised by their gregarious habit. *Eichhornia crassipes* is a free floating and fresh water plant, whereas *Monochoria hastæfolia* is a sub-aquatic herb which occurs in marshy places and on the sides of tanks and 'jhils' and remains attached to the soil by means of a subterranean rootstock. The plants often attain a height of five to seven feet during the rains and have a tendency of adjusting their height with the rise of the water level as seen in some deep water paddy. The inflorescence is always borne above water.

The family Pontederiaceæ has not received much attention from morphologists and cytologists. Smith (1898) first gave an account of the development of seed in *Eichhornia crassipes* and *Pontederia cordata* and also figured the female gametophyte of *Heteranthera graminis*. Coker (1907) has also described the development of the female gametophyte and endosperm in *Pontederia cordata* and *Heteranthera limosum*. His observations on *Pontederia* were confirmed by Smith (1908). Palm (1920) has described the development of pollen grains in some members of the family. Juliano (1931) has recently given an account of the morphology of *Monochoria vaginalis*. Ono (1928) has studied the embryology of *Monochoria Korsakowii*, *M. vaginalis* and *Eichhornia crassipes*. He recorded for the first time a peculiar type of endosperm development in the two species of *Monochoria* he studied. Parija (1934) has carried out some physiological investigations on *Eichhornia crassipes* and Banerji and Ganguly (1937) have studied the mode of spermatogenesis in the same plant.

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Material and Methods

The material used in this investigation was collected from marshy places in the suburbs of Calcutta. Flower buds, styles and ovaries in various stages of development were fixed in the following fixing fluids :— La Cour's 2BE, Allen's modified Bouin's fluid and Licent's fluid. As a rule fixation was done on bright sunny days between 8 A.M. and 2 P.M. The usual methods of washing, dehydration, clearing and embedding were followed. Sections were cut 8 to 16 microns thick depending on the material and the stage required for study. Heidenhain's iron-alum hæmatoxylin with or without Orange G as a counterstain was chiefly used. Newton's Iodine Gentian violet was also tried.

For the study of spermatogenesis *in vitro* pollen grains were artificially germinated on slides containing a thin film of dried agar-sugar solution. Slides were fixed and stained in the usual way.

General Morphology

The stem of *Monochoria hastæfolia* is a subterranean rootstock of spongy texture and remains covered by the remnants of old leaf sheaths. Adventitious roots appear from the lower surface of the stem. The leaves are radical. The petioles are long (varying from 16 to 28 inches), sheathing at the base and bear parallel veined leaves which show the characteristic hastate form. The apices of the full grown leaves are acute and the bases are somewhat rounded. The inflorescence is encased by a bract, which is again enclosed by the flattened petiole of the topmost leaf. The long pedicelled flowers are arranged spirally on a short, thick and spongy stalk and anthesis takes place from the top towards the base, *i.e.*, centrifugal type of flowering prevails. The inflorescence curves downwards after flowering is over, but does not come under water as in *Eichhornia crassipes*.

The flowers have an attractive blue colour, they are long pedicelled and show a trimerous symmetry. The perianth leaves are six in number, the lobes are distinct and subequal. Stamens six, adnate to the base of the perianth lobe, one is larger and has the filament toothed at one side. It has a violet tinge. The other five stamens are yellowish in colour. Comparative measurements of the stamens show that the average length of the filament of the biggest stamen is 7·3 mm. whereas of the others is 5·6 mm. The length of the anther varies likewise. The anthers are basifixed. Dehiscence is by terminal pores which later extend downwards. The ovary is syncarpous, superior, three locular and many ovuled. Placentation is axile. The style is long. It has three longitudinal canals extending up to the ovarian chamber. The stigma has three small lobes with hair-like

processes. The fruit is a loculicidal capsule which remains enclosed by the persistent, twisted and withered perianth. Seeds are small and are more or less ellipsoidal in outline. The outer coat is marked with furrows and ridges and is brown in colour.

Flowering commences from the month of May and extends up to the advent of winter, with occasional breaks. If the winter be late then flowering extends even up to the month of November. As a rule no flowers are produced during the winter and the upper ground parts of the plant die down during this season. If, however, the plants are kept in water, shoots appear from the rootstocks but they are stunted in growth and do not produce flowers.

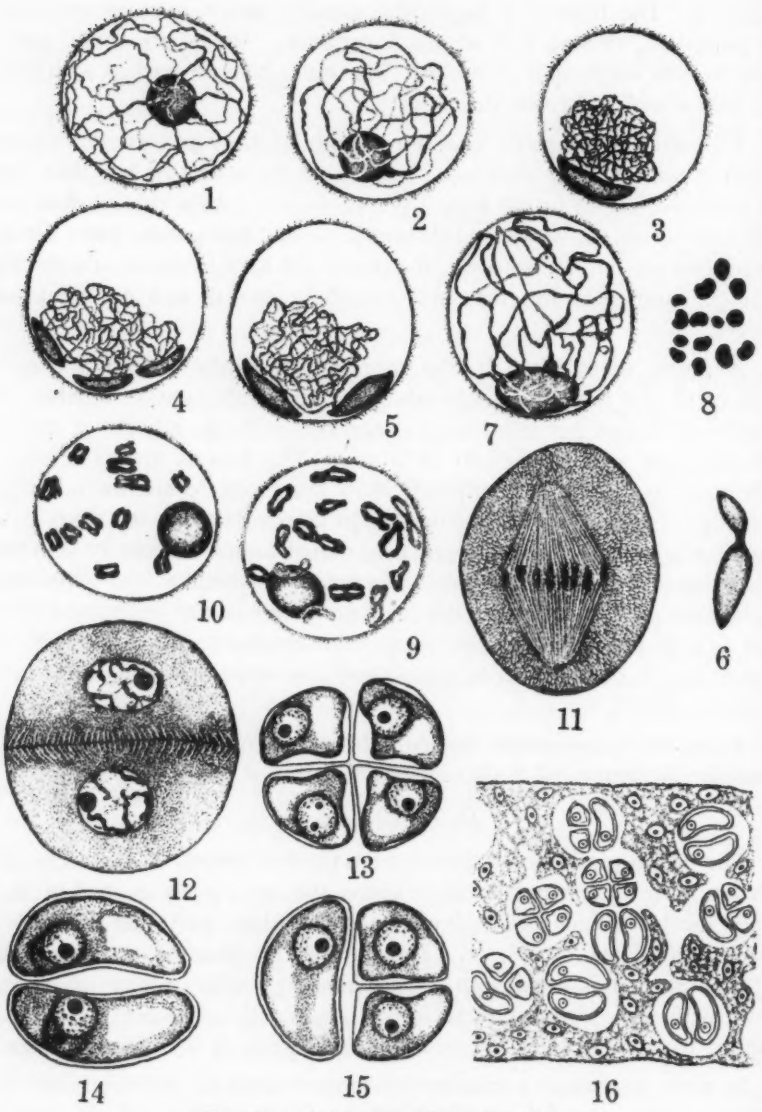
Anthesis takes place in the morning, generally before 8 A.M. The opening of the flowers is delayed by cloudy and humid weather. The number of flowers per inflorescence increases with the advent of the rains and decreases with the advent of winter. The flowers are as a rule self-pollinated, as bagging experiments show that seed formation takes place normally. Generally it takes from ten to fifteen days for the seeds to develop after syngamy. Seeds were germinated under laboratory conditions and germination was also observed in tanks in which a few experimental plants were grown. As a rule, the seeds germinate under water, and it takes from five to ten days for the plumule to emerge from the sheath. The germination is of the " Palm type " and our observations agree with that of Parija (1934).

Vegetative propagation appears to be extensive in this plant and is primarily the cause of " associations " that are commonly seen.

Microsporogenesis

The pollen mother cells arising from the last mitosis of the archesporial cells are more or less polygonal in shape and are closely packed inside the anther loculus. Each cell is bounded by a thin wall and is filled with densely granulated cytoplasm. The nucleus is spherical and occupies a central area and is filled with a peripheral granular matter which stains lightly with basic dyes. The nucleolus is situated in the centre and is surrounded by a clear zone. A small nucleolar bud is seen at this stage.

In early prophase a number of delicate threads are seen inside the nuclear cavity. Careful examination reveals the nature of the leptotene threads which at certain regions are seen to be distinctly double (Text-Fig. 1). The individuality of the threads could not be made out at this stage, but the connection of one of the threads with the bud-like protrusion of the nucleolus is apparent (Text-Figs. 1 and 2). The chromatic threads soon recede



TEXT-FIGS. 1–16. *Monochoria hastæfolia*

Fig. 1. Early prophase. ×2180. Fig. 2. Beginning of first contraction. ×2180. Figs. 3–5. Synizesis. Note the number and form of the nucleolus. ×2180. Fig. 6. A deeply constricted nucleolus at synizesis. ×2180. Fig. 7. Pachynema. ×2180. Fig. 8. Polar view of I-division showing 14*n* chromosomes. ×2180. Fig. 9. Diplonema. ×2180. Fig. 10. Diakinesis. Note connection of a pair of bivalents with the nucleolus. ×2180. Fig. 11. I-division. ×1626. Fig. 12. Interkinesis. Note cell plate at centre. ×1626. Figs. 13–15. Different forms of pollen tetrads. ×932. Fig. 16. Periplasmodium. ×286.

away from the periphery of the nuclear membrane and a tight knot is produced. The nucleolus bcomes flattened and is somewhat lens-shaped and lies close to the nuclear membrane, but it is never enclosed in the meshes of the threads (Text-Fig. 3). In a few preparations two or more such nucleoli have been noted (Text-Figs. 4 and 5). It is interesting to note that working on *Lathyrus odoratus*, Latter (1926) observed a similar form of the nucleolus at synizesis. Definite evidence regarding the connection of the nucleolus with the threads was not obtained at this stage due to the close nature of the synizetic knot. The paired condition of the threads become more evident during the opening of the synizetic knot. This stage, unlike the previous one, is of short duration. At the pachynema stage the threads become thicker and a distinct connection of a pair of threads with the knob-like protrusion of the nucleolus is clearly seen (Text-Fig. 7). The nucleolus recovers its original form but appears to be somewhat ellipsoidal. The pachytene threads overlap one another in all directions and give the appearance of a continuous spireme (Text-Fig. 7). It is at this stage that the pollen mother cells separate from one another and round off.

Transition from the pachynema to the diplonema stage was observed in a very few preparations. The pachytene threads undergo contraction and become thicker. The paired threads appear to open out at certain places but they are kept in contact on account of the formation of chiasma. On account of the small size of the chromosomes, the quadruple nature of the bivalents could not be made out, but that each chromosome is composed of two chromatids could be inferred from the wavy outline of the chromosomes (Text-Fig. 9). Contraction of the pairing homologues proceeds rapidly and at diakinesis they become compact in form and lie close to the nuclear membrane. The members of a bivalent pair are mostly in the form of short rods. One bivalent pair is always seen attached to the nucleolus which occupies a somewhat peripheral position (Text-Fig. 10). The nucleolus gradually becomes smaller in size and paler in appearance and finally it disappears. It is significant to note that the nucleolus maintains a spherical form till its disappearance by progressive reduction in size.

In the prometaphase stage the nuclear membrane becomes very faint and at last it disappears. The chromosomes which have become very much condensed approach towards the centre. At metaphase the chromosomes are aligned on the equatorial region of the spindle (Text-Fig. 11). A polar view of an equatorial plate shows clearly 14 chromosomes (Text-Fig. 8). Three of the gemini appear to be smaller in size. The anaphasic movement of the chromosomes appears to be quite regular. On reaching the poles the chromosomes at first come close together but they move apart and a

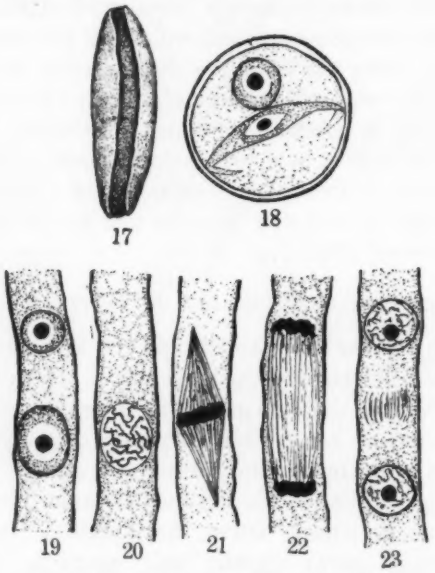
nuclear membrane is secreted. At this stage the protoplast assumes a phragmoplastic appearance and a hyaline membrane is noted in the central region of the spindle which gradually thickens and the two nuclei become separated by a cell plate (Text-Fig. 12). An interkinetic stage is passed through before the second division takes place. At this stage the chromosomes show their individuality and are mostly dispersed towards the nuclear membrane. They are somewhat elongated in form. The second division follows very soon. The spindles vary in their relative orientation and spatial arrangement, they may be parallel or at right angles to one another. During anaphase the sister halves of the chromosomes move regularly to the poles. In many preparations 14 chromosomes have been noted at this stage also. At telophase four nuclei are organised which show the presence of nucleoli and fine chromatin threads. The nuclei are separated by cell plates. The usual arrangement of the tetrads is isobilateral (Text-Fig. 13), but arrangements as shown in Text-Figs. 14 and 15 also appear to be very common. It is interesting to note that such modes of arrangement of the pollen tetrads have also been noted in *Eichhornia crassipes*. The young microspores are separated as a result of the splitting of the cell plates and at first have a somewhat shrunken appearance. Very soon they round up and the exine becomes differentiated. The pollen grains at this stage are uni-nucleate, the nucleus being situated at the periphery and a big vacuole lies at the centre of the cell.

At an early stage in the development of the microspores the tapetal cells become sharply delimited from the sporogenous cells and show the presence of a single nucleus in each cell. The nuclei soon divide and the cells become binucleate. During the synizetic stage of the pollen mother cells, the walls delimiting the individual cells disappear and the tapetal cells protrude inside the anther cavity from all directions. At diakinesis the plasmodium grows further inwards and the nuclei which lie embedded in the cytoplasm migrate in between the pollen mother cells. Gradually the entire cavity of the microsporangium becomes filled up with the plasmodial substance in which the pollen tetrads appear to lie embedded (Text-Fig. 16). With the liberation of the microspores from the tetrad, the plasmodial substance is gradually absorbed and the nuclei degenerate.

Pollen Grains and the Development of the Male Gametophyte

Examination of the pollen grains in methylene-green-gelatin as advocated by Wodehouse (1935) shows the occurrence of fine granulations all over the surface, and the presence of two furrows situated on diametrically opposite sides (Text-Fig. 17). The mature pollen grains are elongate when

dry, but become ovoid when mounted in a liquid medium. Mounted in water and lactic acid the grains burst and eject out their contents. The grains are light-yellow in colour and measure on an average 61·71 μ (length) by 21·38 μ (breadth) when dry. They are binucleate. The vegetative nucleus is larger and occupies a more or less peripheral position while the generative nucleus lies close to it and is embedded in a spindle-shaped cytoplasmic sheath, the ends of which are drawn out (Text-Fig. 18). Smith (1898), and also Banerji and Ganguli (1937) have noted similar form of the generative nucleus in the pollen grains of *Eichhornia crassipes.*



TEXT-FIGS. 17-23. *Monochoria hastæfolia*

Fig. 17. A pollen grain (dry). ×460. Fig. 18. Section of a mature pollen grain. Note the cytoplasmic sheath of the generative nucleus. ×420. Fig. 19. The vegetative and the generative nucleus inside the pollen tube. ×790. Figs. 20–23. Stages in the division of the generative nucleus inside the pollen tube. ×790.

Experiments conducted on the germination of pollen grains *in vivo* and *in vitro* show that the pollen grains absorb moisture and become ovoid in form before germination. Germination normally takes place within ten minutes after pollination and not more than a single pollen tube has been observed to come out of a pollen grain, nor were any branched tubes observed. In artificial media some spirally coiled pollen tubes were seen. Pollen grains obtained from the biggest anther as also from the other anthers showed no difference in germination tests or in shape and size.

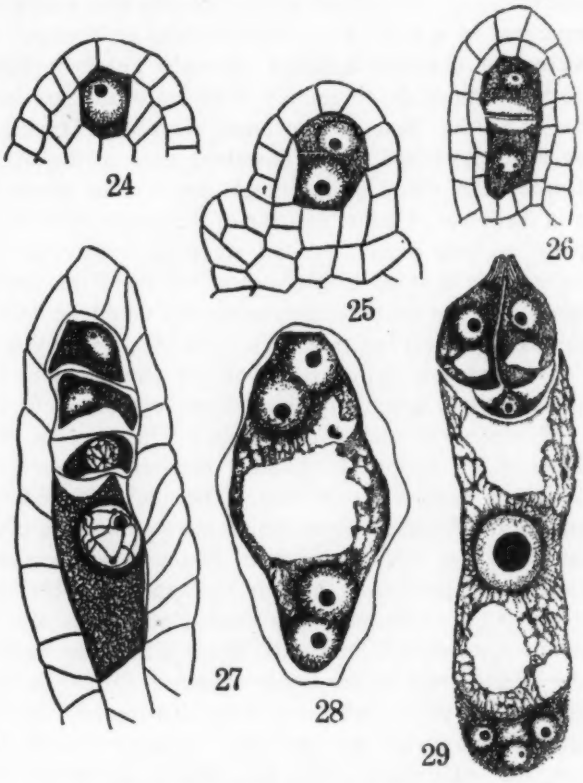
Pollen grains on germination on the stigma give out pollen tubes which take a sinous course and enter the stylar canals, which are lined by conducting cells. The vegetative nucleus passes first inside the pollen tube followed by the generative nucleus. They could be easily made out on account of their differences in size (Text-Fig. 19). The cytoplasmic sheath of the generative nucleus is not apparent at this stage. The generative nucleus soon undergoes division. At prophase a number of elongated bodies —the chromosomes—are seen inside the nucleus (Text-Fig. 20). Soon the nuclear membrane disappears and a well-defined metaphase spindle is organised, in which the chromosomes are aligned in the equatorial region (Text-fig. 21). The anaphasic movement of the chromosomes appears to be regular. On reaching the poles the chromosomes at first pass through a '*tassement polaire*' stage as in normal mitosis (Text-Fig. 22), but soon they move apart and a nuclear membrane is secreted. In late telophase the two nuclei are connected by cytoplasmic striations which disappear later. No evidence of cell plate formation or constriction has been obtained. The two generative nuclei lie very close together and appear to be bereft of any cytoplasmic membrane (Text-Fig. 23).

Development of the Ovule and the Female Gametophyte

The ovules first make their appearance as tiny papillate processes on the placenta. The primordia of the integuments appear very soon and are generally noted after the differentiation of the megaspore mother cell. At this stage the ovules are erect. The curvature of the ovules takes place after the I-division of the megaspore mother cell, and by the time the megaspores are produced the ovules assume an anatropous form. At this stage the integuments almost cover the nucellus. In the later stages the outer integument grows rapidly and envelops the inner. In the mature ovule the micropyle is formed by the two integuments and is straight. Both the integuments are composed of two layers of cells, except the tip of the outer integument which is composed of four layers.

The archesporial cell is hypodermal in origin (Text-Fig. 24). It divides to give rise to a parietal and the sporogenous cell, which later functions as the megaspore mother cell (Text-Fig. 25). Thus the megaspore mother cell is first noted in the third layer of the nucellar tissue. The megaspore mother cell increases in size before prophasic changes commence in the nucleus. After the first division the nuclei become separated by a wall (Text-Fig. 26). The second division soon follows and results in a linear tetrad of megaspores (Text-Fig. 27). The megaspores degenerate from above downwards and the chalazal one always becomes functional. By three successive divisions it

gives rise to an eight nucleate embryo-sac. During these stages the surrounding nucellar cells get crushed and degenerate on account of the pressure exerted by the growing embryo-sac.



Text-Figs. 24–29. *Monochoria hastæfelia*

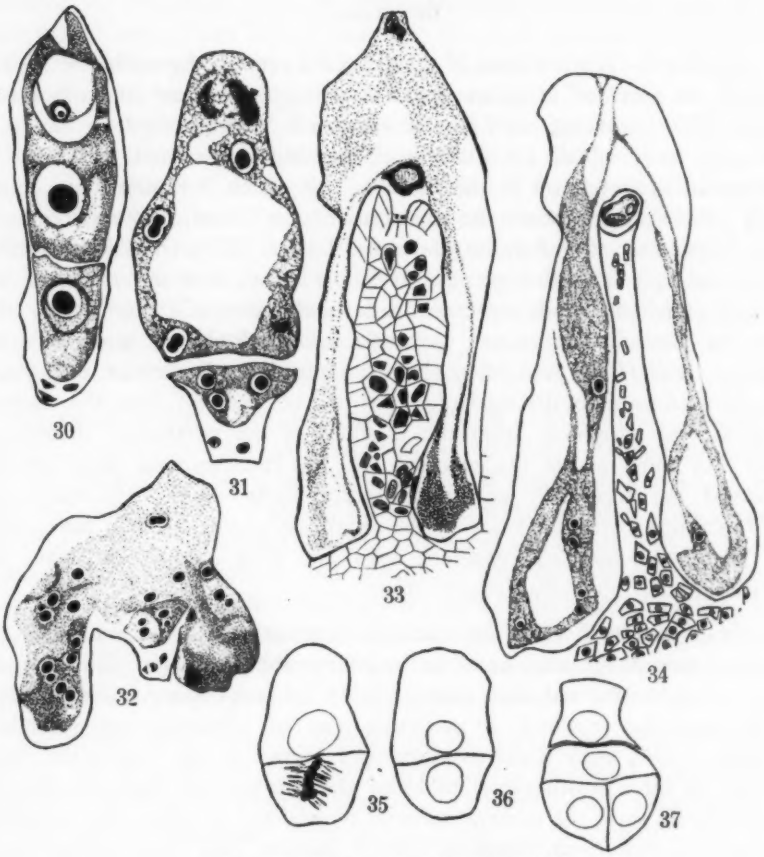
Fig. 24. Hypodermal origin of archesporial cell. ×1032. Fig. 25. The parietal cell has been cut off. ×1032. Fig. 26. A dyad. ×612. Fig. 27. A linear tetrad of megaspores. ×1032. Fig. 28. A four-nucleate embryo-sac. ×1032. Fig. 29. A mature embryo-sac. ×2180.

The mature embryo-sac is broad at the micropylar end. Its length is about 30·4 microns. The synergids are pear-shaped structures with vacuoles at the base and nuclei above them. Distinct filiform apparatus appears to be present. The egg lies below the synergids and has a vacuole at the top. The secondary nucleus lies near about the centre of the embryo-sac. The antipodals are not organised as definite cells but consist of three nuclei which degenerate very soon (Text-Fig. 29).

Development of the Endosperm and Embryo

The primary endosperm nucleus increases in size and undergoes a period of rest before commencing activity. It lies at the centre of the embryo-sac and undergoes division. As a result of the division, two nuclei are produced which are separated by a wall. Thus two chambers are formed, of which the upper or micropylar chamber is larger than the lower or basal chamber. At this stage the antipodals commonly degenerate and are noted as dark shapeless masses at the base of the basal chamber (Text-Fig. 30). The nuclei of the micropylar and basal chambers next divide by free nuclear division and produce as many as six to eight nuclei in the upper chamber and three to four in the lower. Further division of the nuclei of the lower chamber does not appear to take place. At this stage the embryo-sac increases in size and a big vacuole is noted in the central region of the upper chamber. In some preparations the disintegrating synergids could be seen at the top and the antipodals below (Text-Fig. 31). The ovule increases in size still further and the micropylar chamber now shows a tendency to bulge out at the two sides. The two arms containing dense cytoplasm and many free nuclei. Fig. 32 represents such a condition. This process of elongation of the two arms of the micropylar chamber becomes very pronounced and a tuning-fork like appearance is presented. The nuclei of the basal chamber lies at the top of the nucellar tissue which stands out as a column in the central region (Text-Fig. 33). On account of the rapid increase in size of the ovule as well as the protruding arms of the upper chamber, big vacuoles are noted in the latter. Gradually the basal chamber as also the central nucellar tissue disorganise, and the central space is filled up by the extension inwards of the lateral arms of the upper chamber (Text-Fig. 34). It now contains many nuclei which are irregularly distributed. Wall formation begins first at the micropylar end and then extends towards the chalazal region. When the endosperm cells are finally delimited they show a polygonal outline and contain a single nucleus with one or more nucleoli.

The fertilised egg undergoes a period of rest and divides long after the division of the primary endosperm nucleus. The oospore increases in length before division and the first division is by a transverse wall resulting in the production of a terminal and a basal cell (Text-Fig. 36). The basal cell divides again to produce a three-celled proembryo. In some ovules the terminal cell divides again by a periclinal wall resulting in the production of a four-celled proembryo. The terminal cell of the three-celled pro-embryo next divides by an anticlinal wall (Text-Fig. 37). Later stages obtained show a globular embryo in which the dermatogen has been clearly marked off. Due to rapid cell divisions in various planes the embryo becomes elongated



TEXT-FIGS. 30–37. *Monochoria hastæfolia*

Fig. 30. The micropylar and the basal endosperm chambers. ×790. Fig. 31. The enlargement of the micropylar chamber and the multinucleate condition of both the chambers. ×790. Fig. 32. The downward extension of the lateral arms of the micropylar chamber. ×465. Fig. 33. The two lateral arms of the micropylar chamber have reached the chalazal end. Note the degenerating basal chamber at the top of the nucellar column. ×225. Fig. 34. The disintegration of the cells of the central column and the inward extension of the lateral arms. ×225. Figs. 35–37. Some early stages in the development of the embryo. ×775.

and a notch appears at one side. The cells of this region show meristematic activity. This is the origin of the stem tip. In the mature seed the embryo does not fill up the entire space but is enclosed by the endosperm cells, the reserve matter of which seems to contain some crystalline structures which stain deeply.

Discussion

Cytology.—The presence of a specialized area in the nucleolus to which threads are attached in synizesis and later stages has been first observed by Latter (1926) working on *Lathyrus odoratus.* She referred to this as the nucleolar body which acted as an elaborating organ and transferred the elaborated materials on to the threads with which it remained in contact. Such relationship between the nucleolus and a ' loop of the spireme ' has since been observed in *Lathrea* (Gates and Latter, 1927), *Oenothera* (Sheffield, 1927) and in a few other plants. It is now known that this nucleolar body with its attached threads represents a particular pair of chromosomes which have the power of organizing the nucleolus at telophase. Some observers, notably Heitz (1931) and others have noticed the attachment of a pair of Sat-chromosomes to the nucleolus and are of opinion that the nucleolus originates at telophase from the satellites of the particular chromosome pair. In some plants the presence of Sat-chromosomes have not been observed but secondary constricted chromosomes occur. In recent years the functional correspondence of the secondary constriction to the satellites has been recognised and generally speaking such constrictions have been referred to as ' nucleolar constrictions '.

In *Monochoria hastæfolia* definite connection of the nucleolus with a pair of threads has been noted in the later prophasic stages. During pachynema a single spherical dark staining body is always found lying at the side of the nucleolus to which a particular pair of chromosomes seem to be attached. This body which is commonly known as the "nucleolar body" has not the same position in all material where its presence has been detected. Im *Lathyrus*, as in the present material its position is apparently superficial, whereas in *Oenothera*, Sheffield (1927) states—" the body appears to be towards the periphery of the nucleolus, it does not usually project from the surface ". At diakinesis a pair of bivalents are seen to be attached to the nucleolus, no satellites could be seen in this pair, but the close association from prophase to diakinesis leads one to infer that this particular set is endowed with the property of organizing the nucleolus, and it is likely that the nucleolus organizing body is located at the end of this pair of chromosomes as has been noted by Nandy (1937) in Rice.

The flattened or convex form of the nucleolus in synizesis and its position close to the nuclear membrane has also been observed by Latter (1926) in *Lathyrus odoratus.* She thinks that by the presentation of a large area of the nucleolus to the cytoplasm, absorption of a certain portion of the inflowing cytoplasmic substances is possible. This substance is later

elaborated by the nucleolar body which transfers elaborated material on to the thread with which it is in contact. Evidence obtained in the course of this investigation, however, does not favour such a hypothesis. It appears that the lens-shaped form of the nucleolus in synizesis is due mainly to the effect of the fixing fluid. It is a well-known fact that synizesis represents a very delicate condition of the nucleus where the threads are ' balled up ' on account of the action of the fixing fluid. In this material, it appears that the nucleolus is also markedly affected at this stage and becomes biconvex in form. Sometimes it gives rise to two or more lens-shaped nucleoli as a result of constriction induced by the fixing agent (Text-Figs. 4 and 5). Fig. 6 shows a deeply constricted nucleolus which lends support to this view. Further, the typical spherical or ovoid form of the nucleolus in later stages of meiosis adduces additional evidence.

Binucleate pollen grains are commonly seen in this family, as also the spindle-shaped appearance of the generative nucleus. During gametogenesis a well-defined metaphase spindle is seen inside the pollen tube, as has been commonly observed by workers on non-Liliaceous plants. With regard to the process of cytokinesis, neither phragmoplasts with cell plates nor furrows have been observed, and it appears that the two nuclei become separated on the disappearance of the connecting fibres at telophase. Such a condition has been recorded by Maheshwari and Wulff (1938) for a number of plants. Swamy (1941) also did not find a separating membrane or cell wall between the separated male nuclei of *Cymbidium bicolor*.

Formation of a tapetal plasmodium has been recorded by previous workers in this family. Clausen (1927) has described four types of amœboid tapetum in monocotyledons. Following his classification it appears that plasmodium formation in *Monochoria hastæfolia* is of the " *Triglochin* " type.

Embryology.—The development of the female gametophyte in *Monochoria hastæfolia* is of the normal type and corresponds to the accounts given by Ono (1928) for *M. Karsakowii* and *M. vaginalis*, and by Juliano (1931) for the latter plant. None of these investigators, however, mention the presence of a filiform apparatus in the synergid. Regarding the antipodals, Juliano (1931) states, " the antipodals are distinct and they remain perfectly differentiated long after endosperm formation is well under way. They are separated by definite walls and may lie in a single row". Evidence obtained in the course of this investigation shows that the antipodals degenerate before the development of the endosperm and are bereft of any membrane.

The development of the endosperm so far studied in the family Pontederiaceæ indicates that it is of the " Helobiales " type. Coker (1907) who worked on *Heteranthera limosum*, *Pontederia cordata* and *Eichhornia crassipes* states, that in the first two plants the division of the primary endosperm nucleus is followed by a wall, so that as in *Monochoria*, two unequal chambers are produced. In *Heteranthera* the lower chamber shows the presence of a number of endosperm cells which later becomes separated by walls. In *Pontederia* as in *Heteranthera* the development of the endosperm in the upper chamber shows no peculiarities, but in the lower, the nuclei increase in number rapidly by mitotic division and later walls are laid down between them. The number of cells produced is quite large, as many as 200 may be present. Coker (1907) has not studied the development of endosperm in *Eichhornia* in detail, but from the observations he has made, he is of opinion that it is similar in every way to that in *Pontederia* and *Heteranthera*.

Ono (1928) first described the development of endosperm in *Monochoria Korsakowii* and *M. vaginalis*. This was followed by a contribution by Juliano (1931) on the same subject. Ono (1928) found that the development though of the " Helobiales " type showed certain peculiarities. Two lateral haustoria-like processes develop from the base of the upper chamber and consume the nucellar tissue at the chalazal region. Later the upper endosperm chamber, *i.e.*, the portion lying between the two haustorial processes elongates and fills up the entire sac. The basal chamber is thrust into the chalazal region and contains dense cytoplasm with many nuclei, but without any cell walls. Juliano (1931) worked on *M. vaginalis*. He does not refer to Ono's (1928) work, and simply states that the development is of the Helobiales type. But a careful examination of his figures shows that he has missed the stages representing the elongation of the micropylar chamber. Fig. 30 of his paper shows the prolongation of one of the haustorial processes which he does not explain.

The development of the endosperm as observed in the course of this investigation corresponds in essential features to that described by Ono (1928). The main point of difference lies in the mode of disintegration of the central nucellar column, which Ono thinks is brought about by the elongation of the central portion of the upper chamber. Careful examination of a large number of preparations shows that after the two lateral arms have reached the chalazal end, the central nucellar column degenerates. These lateral arms (the so-called haustorial processes of Ono) then grow inwards and finally fill up the entire central space. The basal endosperm chamber along with its nuclei degenerate and is generally absorbed. We have not been

able to observe the disintegration of the haustorial processes as mentioned by Ono.

Summary

The paper gives an account of the morphology, cytology and embryology of *Monochoria hastæfolia.*

1. *Monochoria hastæfolia* is a marshy plant. It has a subterranean root-stock and bears hastate leaves. The flowers show trimerous symmetry. Of the six stamens, one is larger and the anther is differently coloured. The pollen grains produced in this anther show no morphological or size-difference when compared to those produced in the other anthers. All are equally viable.

2. Anthesis of flowers takes place generally before 8 A.M. On cloudy and humid days it is delayed. Bagging of inflorescences shows that self-pollination is the rule. Seed formation takes place under natural conditions and it takes about 15 days for the seeds to develop after syngamy. The germination of the seed is of the " Palm-type ".

3. The development of the microspore appears to be of the successive type. During heterotypic prophase the nucleolus becomes adpressed against the nuclear wall and assumes a lens-shaped structure. At this stage a distinct connection of the nucleolus with the spireme is first noted. At pachynema a small spherical body is seen to lie at the side of the nucleolus to which the spireme is connected. This has been referred to as the nucleolar body. At diakinesis a pair of bivalents are also seen to lie attached to the nucleolus. The haploid number of chromosome is 14. Cytokinesis takes place by cell plate formation.

4. The pollen grains have an elongated appearance when dry. The exine shows the presence of fine granulations all over the surface and has two furrows situated diametrically opposite to each other. The pollen grains are bi-nucleate at the time they are shed. The generative nucleus lies within a cytoplasmic sheath, the ends of which are drawn out.

5. The pollen grains germinate on the stigma within ten minutes after pollination. The pollen tubes pass through the stylar canals and the division of the generative nucleus takes place inside the pollen tube. The metaphase spindle is well organised. Furrows or cell plates have not been observed and it appears that the sperm-nuclei become separated by the disappearance of the spindle fibres.

6. A single archesporial cell develops in the hypodermal layer of the nucellus. It cuts off a parietal cell and then functions as the megaspore

mother cell. A normal linear tetrad of megaspores is produced of which the chalazal one becomes functional and produces an eight-nucleate embryo-sac. The mature embryo-sac is of the normal angiospermous type. The antipodals are ephemeral.

7. Endosperm formation is of the " Helobiales " type. At an early stage of the development of the endosperm, the micropylar chamber extends at the sides leaving a central column of nucellar cells, at the top of which the basal chamber lies. These micropylar processes reach the chalazal end of the embryo-sac. At this time the central nucellar column and the basal chamber degenerate and the two lateral arms of the micropylar chamber extend inwards and fill up the central space.

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THE BONY PALATE OF *UROMASTIX* MERREM*

By Rameshwar Dayal Saksena, M.Sc.

(*Lecturer in Zoology, Balwant Rajput College, Agra*)

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1. *Introduction*

As is generally recognised, the bony palate of *Sauria* differs remarkably from that of *Rhynchocephalia* in the arrangement of the palatines, pterygoids and prevomers.[1] In the former the pterygoids are separated from the

* In a letter, dated February 8, 1937, Dr. Robert Mertens pointed out to me that the genus should properly be called *Uromastyx* (not *Uromastix*), as that was the original spelling employed. The Rules of Nomenclature, however, state that the original orthography is to be preserved unless a typographical error, an error of transliteration, or a *lapsus calami* is evident. As the spelling in question comes under the heading ' error of transliteration' (Malcolm Smith in a letter to me, dated January 14, 1939), the author prefers to write ' *Uromastix* '.—B. C. Mahendra.

[1] To quote Broom (1935), who sums up the present position about the homology of the so-called ' vomers ' of the Anamnia, " During the last 40 years the view that the anterior so-called vomers of the fish, amphibian and reptile are not true vomers has steadily obtained more and more support, and the name prevomer is now almost universally applied to them in America and Europe. In America Osborn and Gregory were among the first to accept the new view, and so far as I am aware all the palæontologists and anatomists are in agreement. In England Watson and Goodrich have given their whole-hearted support. In Germany v. Huene for a time hesitated but is now also in agreement."—*Annals of the Transvaal Museum*, **18**, Pt. 1, p. 25.

prevomers by the palatines (Williston, 1925; Goodrich, 1930; Versluys, 1936, etc.), while in the latter they extend forwards to meet the prevomers and are apposed in the middle line so as to keep the palatines of the two sides apart from each other. This difference in the palatal region between *Sphenodon* and Lizards, pointed out first by Günther (1867), was specially emphasized by Howes and Swinnerton (1900), who made observations on the development of these bones in *Sphenodon*.

In 1905, however, Beddard, on the basis of superficial observation of a single skull of *Uromastix ægyptius* (= *spinipes*) noted that the pterygo-palatine suture, although obliquely disposed posteriorly, anteriorly " becomes parallel with the long axis of the pterygoid itself, and only *dies away anteriorly close to, if not in actual contact with, the vomers.*[2] If the vomers of that lizard *happened to be rather larger than they actually are* in this species, and as they undoubtedly are in some lizards, *there would be a prolongation forward of the pterygoids to the vomers.* As it is, their forward growth results in the complete severance from each other of the palatines, except possibly for a very minute space anteriorly. "[3]

Although Beddard did not find the pterygoids actually in contact with the prevomers (*vide* his text-fig. 1 and the italicised portions in the foregoing quotation), he stressed the potential possibility of such a state of affairs occurring, provided the prevomers were larger than they are in *Uromastix*, and compared " the forward growth of the pterygoids....very nearly to reach the vomers " in this lizard to the condition found in *Sphenodon*. Beddard's note, partly observational but mostly speculative, couched in dubious language, naturally failed to attract the attention of all later workers on the saurian skull. (Williston, 1925; Versluys, 1927; Goodrich, 1930; Versluys, 1936, etc.)

As the feature in question is of considerable importance from the phylogenetic standpoint, it was suggested to me by Mr. Beni Charan Mahendra that I should scrutinise the various species of *Uromastix* to find out if in any of them there was an actual pterygo-prevomerine contact, comparable to the one hitherto described only in *Sphenodon*.

The *Uromastix* skull, apparently, has not attracted much attention. Hoffmann (1890) has figured the lower jaw of the genus and made one or two passing remarks about the skull, although none on the bony palate. Siebenrock (1895) deals with the skull in his account of the skeleton of

[2] *I.e.*, prevomers.

[3] The italics are mine—R.D.S.

Agamid lizards, but he neither gives a figure of it nor mentions the palatal peculiarities with which we are concerned here. Busch (1898) has described the palatal region in *Uromastix hardwickii*, but his description, agreeing as it does with the general saurian arrangement, does not correspond actually with the skull, although I have examined skulls of a great many individuals. Beddard (1905), in the paper already mentioned, deals with *Uromastix ægyptius* (= *spinipes*), while Sedgwick (1905) gives a figure[4] of the skull of the genus, without mentioning the species figured.

2. *Material and Technique*

Three species of *Uromastix* were examined: *Uromastix acanthinurus* Bell,[5] *U. ægyptius* Forskål (= *U. spinipes* Daud.)[5] and *U. hardwickii* Gray. An identified specimen each of the former two species was presented by Dr. Robert Mertens, Natur-Museum Senckenberg, Frankfurt a.M. (Germany) in 1936 for the present investigation to Mr. Mahendra, who kindly passed them on to me this year. Both the individuals were fully grown, and measured as follows :

	From snout to vent	Tail
U. acanthinurus	14·3 cm. ..	9·5 cm.
U. ægyptius	18·7 cm. ..	12·6 cm.

The specimen of *U. acanthinurus* originally came from Algeria and that of *U. ægyptius* from Egypt.

Over a dozen specimens of *U. hardwickii* were collected for the present study in the suburbs of Agra ; they included various-sized individuals of both sexes.

The following methods were used:

(*a*) *Preparation of skulls by maceration.*—After removing the skin from the heads and keeping them in a 1 per cent. solution of caustic potash for two or three days, the muscles were carefully scraped off, and the cleaned

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[4] Fig. 186 on page 341. The figure is made by Mr. J. J. Lister, F.R.S.

[5] For the following taxonomic information regarding these two species, I am indebted to Dr. Robert Mertens :—

" *Uromastyx ægyptius* stammt von Forskål 1775, *U. spinipes* von Daudin 1803. Den Namen *ægyptius* (an Stelle von *spinipes*) hat bereits Anderson in *Zool. Egypt Rept. Batr.*, p. 129, 1898 angewandt. Doch gibt er als Autoren von *ægyptius* Hasselquist und Linné 1757 an, was nicht richtig ist, da unser modernes Namensystem erst mit der 10. Aufl. von Linné's *Systema naturæ* beginnt, die im Jahre 1758 erschien ist. Flower (*Proc. Zool. Soc.*, London, 1933, p. 779) erwähnt als Autor von *U. ægyptius* richtig Forskål 1775.

" *Uromastyx acanthinurus* ist von Bell im Jahre 1825 aufgestellt "—B. C. Mahendra.

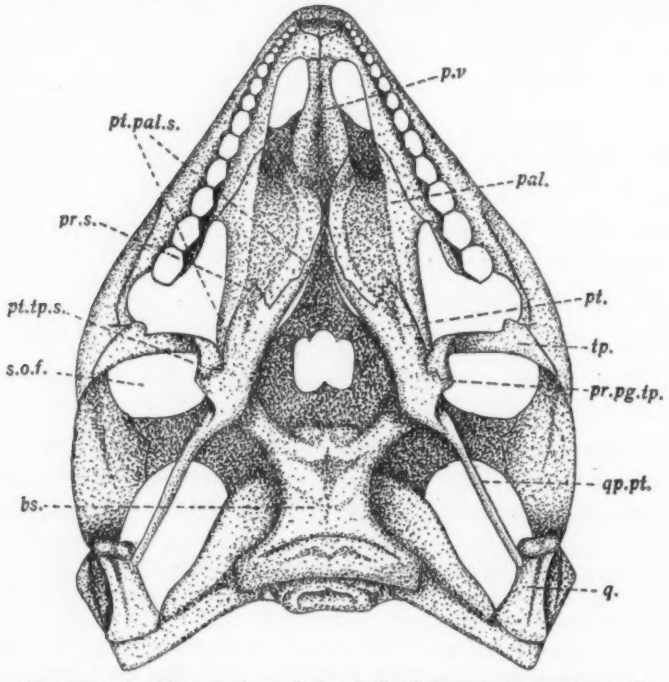
preparations treated with hydrogen peroxide. The component bones were separated from each other by boiling.

(*b*) *Alizarin-stained Preparations.*—These were made according to the process described by Mahendra (1936). It was found that the bony palate, cut off from the adjacent bones and mounted in glycerin, showed the sutures very distinctly under a microscope.

(*c*) *Sections.*—Skulls for cutting sections were decalcified in Jenkin's fluid, stained in Borax carmin, dehydrated, cleared in xylol and embedded in paraffin. Serial sections, longitudinal and transverse, 15μ thick, were examined.

3. The Bony Palate as a Whole

The floor of the skull in *Uromastix* (Text-Fig. 1), as in other Reptiles, is formed of three regions, the premaxillo-maxillary, the palatal and the

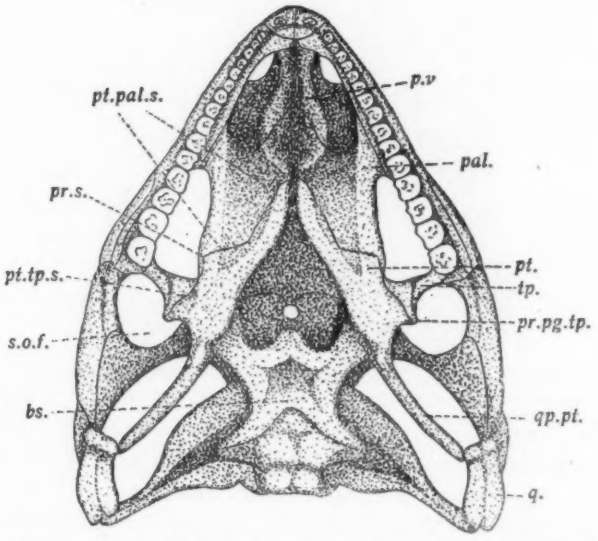


TEXT-FIG. 1. Ventral view of the skull of *Uromastix ægyptius*. ×3.

bs., basisphenoid; *pal.*, palatine; *pr.pg.tp.*, ventral process on the pterygoid and transpalatine, playing in front of the processus mesentericus of the mandible; *pr.s.*, forwardly directed projection on the pterygo-palatine suture; *pt.*, pterygoid; *pt.pal.s.*, pterygo-palatine suture; *pt.tp.s.*, pterygo-transpalatine suture; *pv.*, prevomer; *q.*, quadrate; *qp.pt.*, quadrate process of the pterygoid; *s.o.f.*, suborbital fossa; *tp.*, transpalatine.

basioccipitosphenoidal. A number of vacuities covered with tough membrane are present. Of these regions, the palatal consists of four pairs of bones: *pterygoids*, *palatines*, *transpalatines* and *prevomers*. All these bones are remarkably flattened so that the palatal vacuities are relatively reduced.

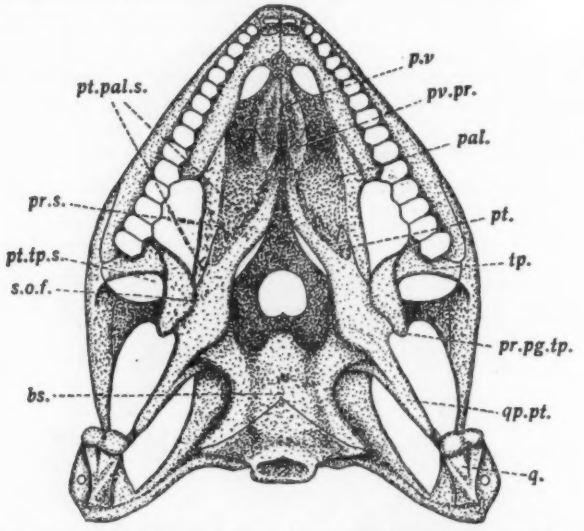
(*a*) *The Pterygo-palatine Suture.*—The suture between the pterygoid and the palatine shows a different condition in each of the three species examined. In *Uromastix acanthinurus* (Text-Fig. 2) it runs forwards, for an



TEXT-FIG. 2. Ventral view of the skull of *Uromastix acanthinurus*. ×3.
Abbreviations as in previous figure.

extremely short distance, from the inner posterior margin of the sub-orbital fossa, then turns obliquely inwards and finally pursues an oblique course mesialward towards the prevomer. In both the other species, the suture in question shows a marked difference from such a relatively simple disposition mainly on account of the presence of a prominent, forwardly-directed process intervening in its course and formed by a backward turning of the suture after the initial extension forwards from the sub-orbital fossa. In *Uromastix ægyptius* (Text-Fig. 1) this process is better developed and the right and left pterygo-palatine sutures approximate towards each other mesially far behind the posterior margin of the prevomer. In *Uromastix*

hardwickii (Text-Fig. 3) the mesial approximation of the right and left sutures is not so marked and a large part of the pterygoids can be discerned extending towards the prevomers.



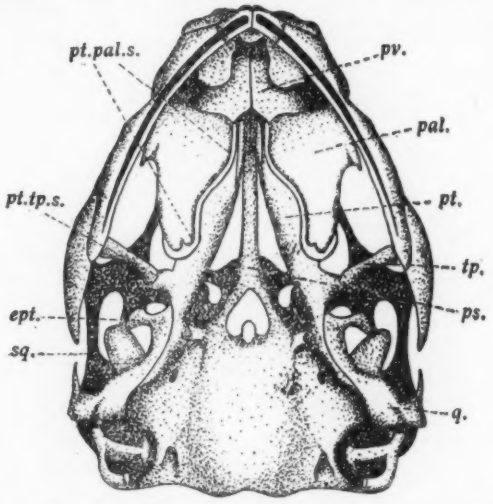
TEXT-FIG. 3. Ventral view of the skull of *Uromastix hardwickii*. ×3.
pv.pr., prevomerine process of the palatine (all other abbreviations as in previous figures).

(*b*) *The Pterygo-transpalatine Suture.*—A comparison of this suture in the three species shows that the inner ventral process of the transpalatine (*vide infra*) in *Uromastix hardwickii* is formed almost exclusively by the transpalatine, while in the other two species the pterygoid also contributes to its formation to a large extent.

(*c*) *The Pterygo-quadrate Connection.*—In *Uromastix hardwickii* the quadrate process of the pterygoid is rather small and stops short of the quadrate, being connected to the latter bone only by a ligament; in *Uromastix acanthinurus* it is slightly longer and extends up to the anterior margin of the quadrate, while in *Uromastix ægyptius* it is as well developed as in other typical saurians (*e.g.*, *Varanus*, *Hemidactylus*, *Scincus*, etc.) and lies closely adpressed to the inner aspect of the quadrate.

(*d*) *The Relation between the Pterygoid and the Prevomer.*—Beddard (1905) laid great emphasis on the extension forwards of the pterygoid towards the prevomer and pointed out its ' similarity ' to the condition found in *Sphenodon*. Careful study, however, not only of cleared alizarin-stained preparations of this region and the structure of the separated bones, but also of serial longitudinal and transverse sections through the bony palate

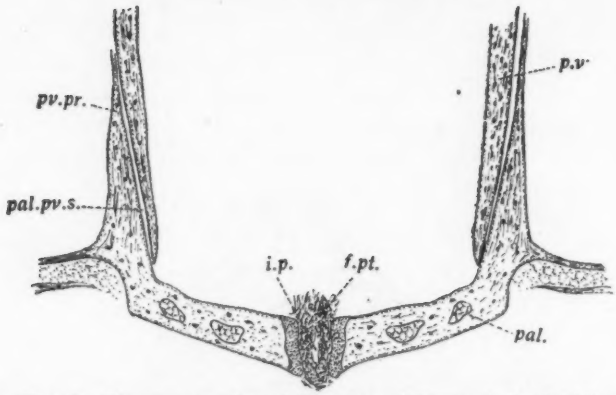
establish beyond doubt that *in no case is the pterygoid in contact with the prevomer*. However, it is remarkable that the arrangement of these bones in *Uromastix hardwickii* very closely resembles that found in stage R (Howes and Swinnerton, 1901, Plate III, Fig. 12) in the development of *Sphenodon* (Text-Fig. 4), a fact which is not easy to account for. While one should be



TEXT-FIG. 4. Ventral view of developing skull of *Sphenodon punctatus*, Stage R (After Howes and Swinnerton, 1901, Plate III, fig. 12, enlarged to 1¾).
ept., epipterygoid; *ps.*, parasphenoid ; *sq.*, squamosal (other abbreviations as in previous figures).

particularly cautious in drawing conclusions from a single fact of this kind, one wonders whether the pterygo-prevomerine contact in adult *Sphenodon*, usually believed to be primitive, should not perhaps prove to be a secondary condition, which originated rather late in phylogeny and was preceded by some such disposition of palatal bones as is hinted at in the development of the Tuatara and still retained in the adult *Uromastix*.

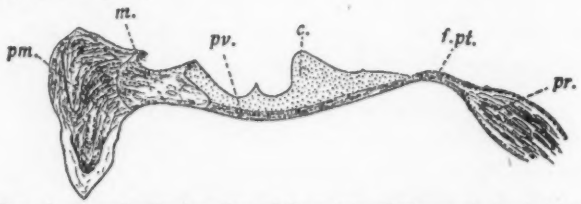
A casual observation of the skull of *Uromastix hardwickii* might give an impression that the pterygoid extends forwards actually to meet the posterior margin of the prevomer, and at first I thought it was actually so. The appearance, however, is spurious, being due to the fact that the part of the palatine immediately in front of the pterygo-palatine suture bears, at its inner ventral aspect, a scallop-shaped process (the *prevomerine process*), to which the prevomer is intimately attached (Text-Fig. 5). It is easy to mistake this process for the prevomer itself. As far as I am aware, such a process has not been hitherto recorded.



TEXT-FIG. 5. Transverse section of the palate of *Uromastix hardwickii*. ×17.

f.pt., fibrous tissue extending forwards from the pterygoid; *i.p.*, inter-palatinal space; *pal.pv.s.*, suture between prevomer and palatine; *pv.pr.*, prevomerine process of the palatine (other abbreviations as in previous figures).

In *Uromastix ægyptius* and *U. acanthinurus* the pterygoids are relatively short and they stop a fair distance behind the posterior margins of the prevomers. In *Uromastix hardwickii*, they are longer and extend farther forwards between the palatines towards the prevomers, although they do not meet them. Their anterior ends are connected to the prevomers by fibrous tissue alone (Text-Fig. 6).



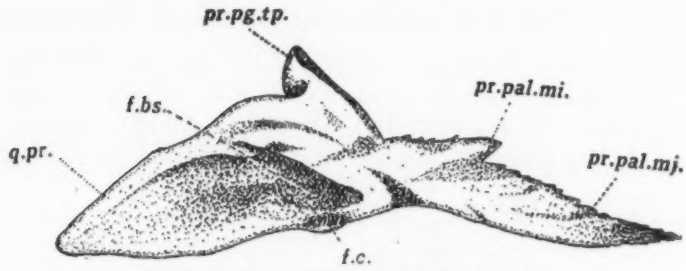
TEXT-FIG. 6. Longitudinal section of the palate of *Uromastix hardwickii*. ×7½.

c., cartilage overlying the prevomer; *m.*, maxilla; *pm.*, premaxilla (other abbreviations as in previous figures).

4. Component Bones of the Palate

As the palatal bones of the two sides are similar, those of only one side need be described in detail.

(*a*) *Pterygoid.*—Each pterygoid (Text-Fig. 7) has "a characteristic appearance and shows differences from that of *Varanus* (Bahl, 1937), *Lacerta Scincus* (El-Toubi, 1938), and other lizards. It is propeller-shaped and is divided by the *fossa columellæ* into an anterior *palatal part*, which is horizontally flattened and participates in the formation of the



Text-Fig. 7. Pterygoid (inner lateral view).

f.bs., facet for articulation with the basipterygoid process of the basisphenoid; *f.c.*, fossa columellæ ; *pr.pal.mi.*, processus palatinus minor; *pr.pal.mj.*, processus palatinus major; *pr.pg.tp.*, ventral process playing in front of processus mesentericus of the mandible; *q.pr.*, quadrate process.

palate, and a posterior part, the *quadrate process*, which is a laterally compressed, plate-like structure directed obliquely upwards and outwards to approach the inner side of the *condylus mandibularis* of the quadrate.

The palatal part, at the place where it joins the quadrate process, shows a ventral process at its outer side, which unites with a process developed on the inner ventral end of the transpalatine and thus forms a prominent projection, not hitherto recorded. This *pterygo-transpalatine projection* is seen to lie just in front of the *Processus mesentericus* of the coronoid, when the lower jaw is tightly closed against the upper. Almost at the same level, but rather towards its inner aspect laterally, there is a facet overhung by a small ridge for articulation with the basipterygoid process of the basisphenoid. There is apparently no *cartilaginous meniscus*, as described for other lizards by Broom (1922).

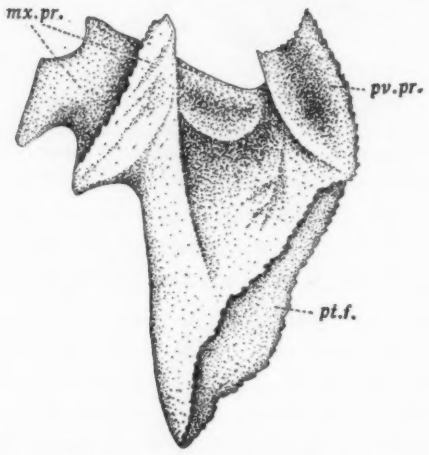
Anteriorly, the palatal part is produced into a smaller, outer (*processus palatinus minor*)[6] and a larger inner process (*processus palatinus major*).[6] The former is highly developed in *Uromastix ægyptius* and a little less so in *Uromastix hardwickii*, but is distinctly rudimentary in *Uromastix acanthinurus*. This disparity in its development is responsible for the different disposition of the pterygo-palatine suture (*vide supra*). The latter process is considerably broad and long in *Uromastix hardwickii*, while it is short and slender in *Uromastix acanthinurus* and *U. ægyptius*.

In none of the species examined does the *processus palatinus major* actually meet the posterior border of the prevomer. There is, however, a fibrous tissue in *Uromastix hardwickii* which runs forwards, in the inter-palatinal space, from the anterior ends of the pterygoids towards the prevomers.

[6] These names are mine, as I do not find either a detailed description of these processes by previous authors or any names employed to distinguish between them.– R.D.S.

The *quadrate process* of the pterygoid has already been described.

(*b*) *Palatine.*—In *Uromastix hardwickii*, the palatine (Text-Fig. 8) is roughly triangular in appearance with the base of the triangle lying anteriorly between the prevomer and maxilla and the apex directed backwards and abutting against the transpalatine. The anterior side of the palatine



TEXT-FIG. 8. Palatine (ventral view).

mx.pr., dorsal and ventral maxillary processes, enclosing the palatine foramen; *pt.f.*, sutural joint for articulation with the pterygoid; *pv.pr.*, prevomerine process.

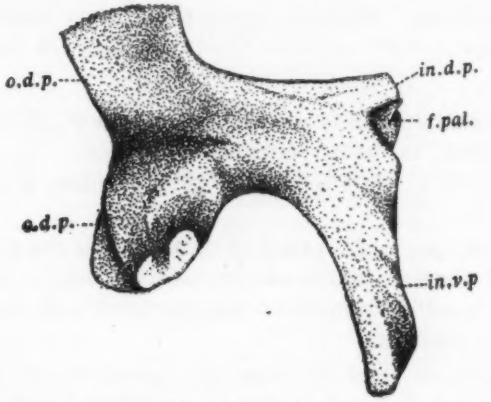
forms the posterior border of the olfactory capsule and bears the *prevomerine process*, already mentioned, on its inner end, while it articulates with the maxilla on its outer end. The inner side, obliquely directed, shows minute notches and processes to dovetail with those of the palatine processes of the pterygoid. The outer side has an antero-posteriorly directed ridge, which extends in the same line as the inner lateral boundary of the sub-orbital fossa and articulates with the inner ventral side of the maxilla. Dorsal to this ridge, there is a lateral process, notched posteriorly and abutting with the maxilla dorso-laterally.

The formation of the "*maxillary*" or *palatine foramen*, through which the *superior alveolar nerve*, the *inferior orbital artery* and the *maxillary vein* pass into the *canalis alveolaris superior* of maxilla, differs in *Uromastix* from that in *Varanus* (Bahl, 1937). While in the latter, the foramen perforates the maxillary process of the palatine alone so that the maxilla takes no part in its formation, in the former it is enclosed between the maxilla on the one hand and the palatine ridge and process on the other.

In *Uromastix ægyptius* (Text-Fig. 1) the inner side of the palatine differs in shape from that in *Uromastix hardwickii* (Text-Fig. 3), as the posterior pterygoid process is very well developed. The bone is considerably broad and forms almost the whole of the anterior palate. The ventral ridge is not so prominent and the prevomerine process is deeply forked so as to accommodate the posterior part of the prevomer between its horns.

In *Uromastix acanthinurus* (Text-Fig. 2) the bone is similar to that in *Uromastix ægyptius*, but the ventral ridge is less developed.

(*c*) *Transpalatine.*—The *transpalatine* (Text-Fig. 9) is similar in its form and disposition in the three species. It is a transversely directed, compact



TEXT-FIG. 9. Transpalatine (viewed from the anterior aspect).

f.pal., facet for attachment with palatine ; *in.d.p.* and *in.v.p.*, inner dorsal and ventral processes; *o.dp.* and *ov.p.*, outer dorsal and ventral processes.

bone, articulating with the pterygoid and palatine at its inner, and with the maxilla and jugal at its outer end. It has a pair of processes at its either end: the *outer* and *inner processes.*

The *outer processes*, a dorsal and a ventral one, are flattened and plate-like and embrace the maxillo-jugal suture completely between them. They are inclined approximately at right angles to each other. The dorsal one, much larger of the two, is disposed horizontally, while the ventral one extends ventro-laterally. The dorsal outer process is notably broader in *Uromastix hardwickii* as compared to that in the other two species.

The *inner processes*, of which one is dorsal and the other ventral, are rather rod-shaped in appearance and accommodate the pterygoid between them, besides being joined by the posterior end of the palatine. Of these,

the ventral one, which comes to lie in front of the *processes mesentericus* when the jaws are closed, has already been mentioned.

The difference in the disposition of the *pterygo-transpalatine suture* in the three species has been noted previously.

(*d*) *Prevomer*.—The prevomer is a scallop-shaped bone similar in the three species. It articulates anteriorly with the premaxilla, and is closely applied to the ventral surface of the *prevomerine process* (*vide supra*) of the palatine. It has no contact with the pterygoid, although, as already mentioned, its similarity and close attachment to the prevomerine process tends to give a false impression of its meeting posteriorly with the anterior attenuated end of the pterygoid. In all the three species, the prevomerine processes of the palatines, although approximating very closely towards their anterior ends, are actually separate from each other at the middle line.

5. Summary and Conclusions

The author has studied the structure of the bony palate and its component bones in detail in three species of *Uromastix* : *viz.*, *U. hardwickii*, *U. acanthinurus* and *U. ægyptius*. The following facts, discovered by him, are new.

(1) The three species show marked differences in the anterior extension of the pterygoid, the form of the pterygo-palatine suture, the relative development of the quadrate process of the pterygoid and the relation of the pterygoid to the prevomer.

(2) The pterygoid does not meet the prevomer in any species. In *Uromastix hardwickii*, however, it shows a condition closely similar to stage R in the development of *Sphenodon*: a fact of great interest.

(3) A process, hitherto not recorded, has been described on the ventral inner end of the transpalatine.

(4) The transpalatine embraces the maxillo-jugal suture between a dorsal and a ventral process, developed at its outer end, so that the formation of the palatine foramen in *Uromastix* differs from that of other Saurian skulls.

(5) The quadrate process of the pterygoid is fully developed in *Uromastix ægyptius*, but it does not reach quite up to the quadrate in *U. hardwickii*. In *U. acanthinurus* it reaches only up to the anterior border of the quadrate.

(6) There is no *cartilaginous meniscus* between the pterygoid and the basipterygoid process of the basisphenoid.

(7) The palatine bears a scallop-shaped *prevomerine process* to which the prevomer is closely applied.

6. Acknowledgments

The problem was suggested to me by Mr. Beni Charan Mahendra, to whom I am extremely grateful not only for continuous help and guidance in the work, but also for passing on to me the specimens of *Uromastix ægyptius* and *U. acanthinurus* which he had obtained from his friend, Dr. Robert Mertens of Natur-Museum Senckenberg, Frankfurt a. M. (Germany). It is a pleasure to thank Dr. Mertens in this connection on Mr. Mahendra's behalf and mine. I am also indebted to Dr. R. K. Singh, Principal, Balwant Rajput College, Agra, for his keen interest and kind support in the work, as well as to Mr. Bahadur Singh for a great deal of encouragement.

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Plates VIII and IX refer to the article on "Life-History and Bionomics of *Pseudococcus saccharicola* Takh. (Homop-Coccidæ)—A New Pest of Sugarcane in India" by Mr. S. Ram Mohan Rao, D.Sc., appearing in Vol. XVI, No. 3.

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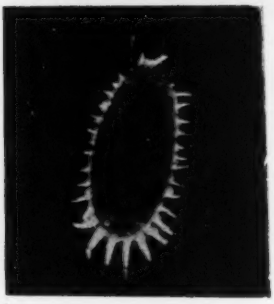

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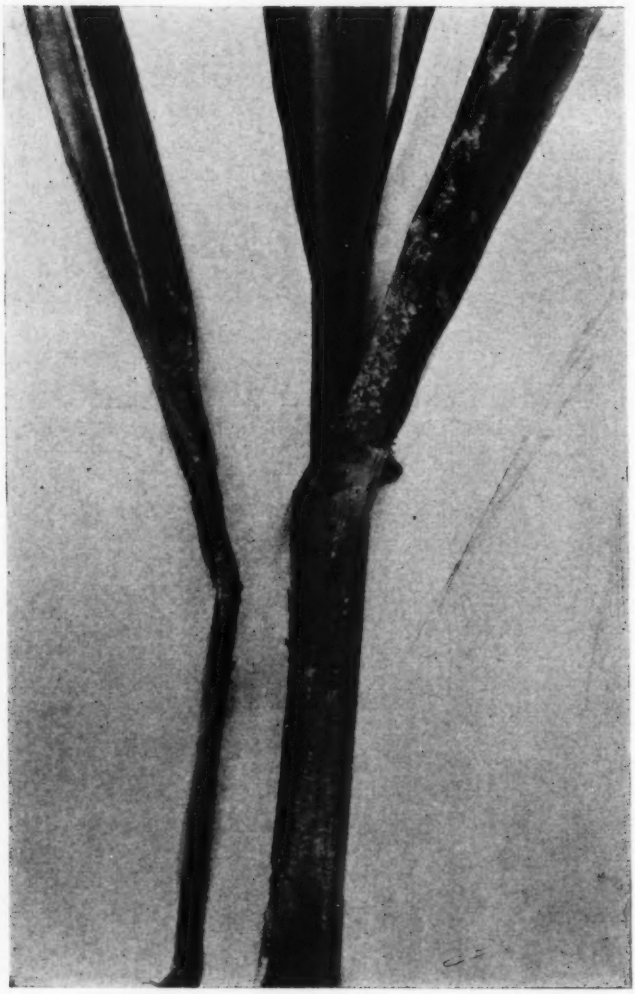


STUDIES IN THE PROTEACEÆ

VII. The Endosperm of *Grevillea robusta* Cunn., with Special Reference to the Structure and Development of the *Vermiform Appendage*

By S. B. Kausik

(*Department of Botany, University of Mysore, Central College, Bangalore*)

Received June 5, 1942

(Communicated by Prof. B. Sahni, M.A., SC.D., F.R.S.)

Introduction

In two recent studies on the embryology of *Grevillea robusta* Cunn. and *G. Banksii* R. Br., the writer (Kausik, 1938 *a* and 1939 *a*) has described the occurrence of a coiled worm-like haustorial structure, the *vermiform appendage*, which develops after fertilization as a part of endosperm from the lower portion of the embryo-sac. A detailed investigation of this structure was very recently made, and the several stages in its development and also its behaviour during seed formation are now given in the following paper. A preliminary note has already appeared some time back (Kausik, 1941).

Methods

The material for this investigation was killed as usual in Bouin's fluid and was subsequently dehydrated slowly in a weak solution of glycerine. After this, chloroform was used for infiltration instead of xylol. Sections were cut ranging in thickness from 16 to 24 μ for younger stages, and from 30 to as much as 40 μ in the case of older material in order to get the endosperm and the *appendage* as completely as possible in one or only a few sections. The sections were all stained in a 0·5% Heidenhain's iron-alum hæmatoxylin. Destaining was generally done in a saturated solution of picric acid (Maheshwari, 1933), but in some cases a 2% iro-nalum solution was also used.

In addition to the microtome sections, many entire preparations of the endosperm were also used for studying the development of the *vermiform appendage*. These preparations were made according to the dissection methods already described elsewhere (Kausik, 1939 *b*). Further, while one

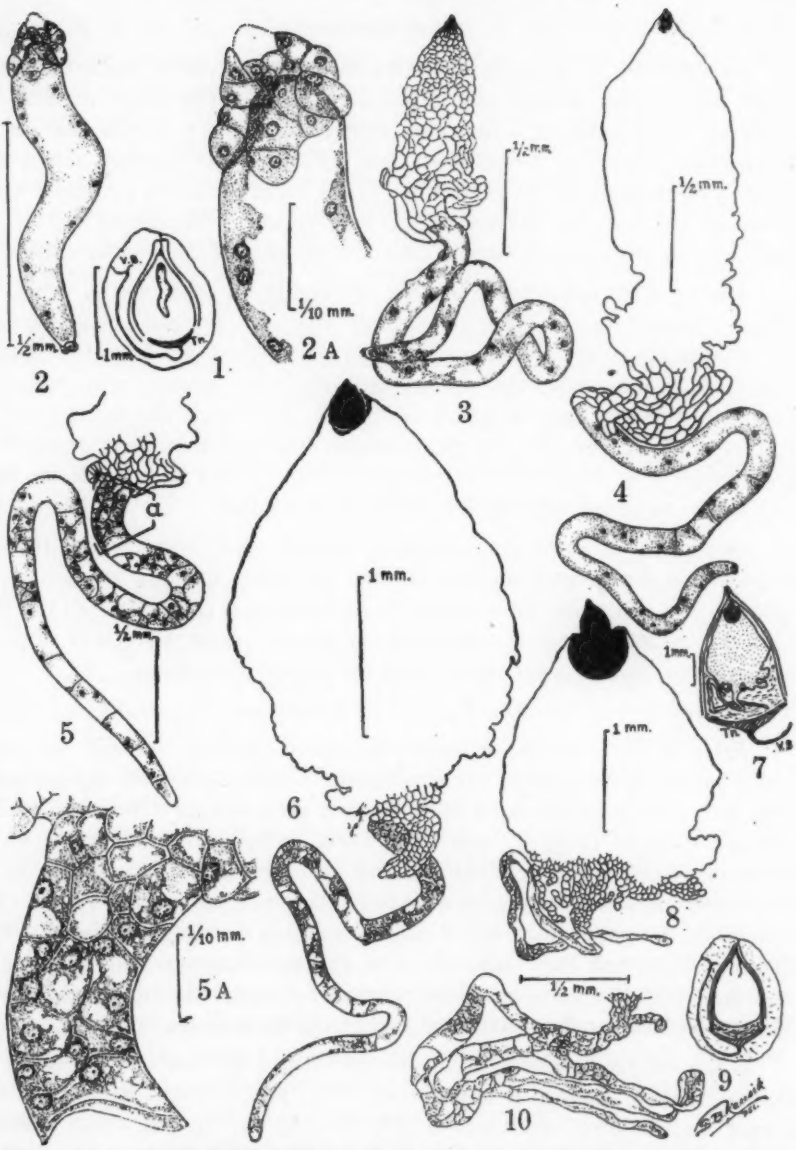
set of such entire preparations were made into permanent whole mounts, after killing and staining, to study the interesting details, another set was used for observing the *appendage* in the fresh living condition as soon as the preparations were dissected out of the seeds.

The Embryo-Sac

The development of the embryo-sac conforms to the normal type (Brough, 1933; Kausik, 1938 *a*). The synergids become definitely beaked in appearance with a clear lateral indentation (Figs. 12 and 13; Pl. X:1), so that the apex in each synergid is distinct from the basal part where a large vacuole is seen. Further, the apex is characterized by the possession of a number of minute striations which converge towards the tip (Fig. 13) to form the "filiform apparatus". The two polar nuclei are found together close to the egg apparatus (Figs. 11-13) and remain without fusing till the actual entry of the pollen tube into the embryo-sac. At the lower end of the sac there are three prominent antipodal cells (Figs. 11 and 15) which persist as such for a long time after fertilization and are seen even during later stages of endosperm formation (Figs. 2, 3, 4, 17, 21, 34, 37; Pl. XII: 12).

The cytoplasm in the embryo-sac is highly vacuolate (Fig. 11; Pl. X: 1) and includes innumerable starch grains which are distributed throughout, except at the micropylar end. This fact is probably to be accounted for as follows: All the cells of the nucellus contain starch, and since the destruction of the nucellar cells begins first all round the lower portion of the embryo-sac (the nucellus being fairly intact at the micropylar region) where again starch is especially abundant, it appears that the starch contained in these cells is passed into the embryo-sac, probably in some diffusible form. The absence of starch in the embryo-sac in the earlier stages of its formation when the surrounding nucellus is but slightly encroached upon seems to lend support to this surmise.

During the final stages of its organization, the embryo-sac enlarges considerably at the expense of the surrounding nucellus, a feature which becomes especially marked later during all subsequent development when the nucellus suffers an almost wholesale destruction on account of a tremendous increase in the size of the sac with the endosperm tissue developing rapidly inside. Nevertheless, even such an increase in size "fails to keep pace with the space vacated by the nucellus" (Brough, 1933), and thus there always remains an unoccupied peripheral region at every stage during the development of the seed.



FIGS. 1–10. Camera lucida drawings of entire living preparations of endosperm of *Grevillea robusta* Cunn. showing the development of the *vermiform appendage*. The *appendage* shows the formation of cross partitions in Fig. 4, and cell organization in Fig. 5 ; Fig. 5*a* is a portion in Fig. 5 (marked *a*) enlarged; some of the cells show two nuclei. In Fig. 6 the cell formation in the *appendage* is more progressive. Figs. 8 and 10. Decline of the *appendage* when it becomes crushed. Figs. 1, 7 and 9 show outlines of seeds in different stages. The embryo is shown black in Figs. 3, 4, 6, 7 and 8. *Tn.*, Pad of tannin tissue at the base of seed : *V.B.*, vascular bundle.

Fertilization

In a number of preparations the pollen tube was seen inside the micropylar part of the embryo-sac, either immediately after entering and discharging its contents, or some time afterwards. Thus it is clear that normal fertilization occurs in *Grevillea robusta*. Further, the entering pollen tube forms a clear canal-like passage (Figs. 17, 18 *c* and 28) in the glandular apical tissue of the nucellus overlying the embryo-sac. Mauritzon (1939) has recorded the presence of such a canal in the case of *Sonneratia acida*.

During fertilization one of the synergids is destroyed, while the other persists for a little time after this. It appears probable that the minute striations at the apex of the synergids which form the *filiform apparatus* have an important rôle in fertilization by secreting some substance and thus directing the course of the pollen tube inside the embryo-sac (*cf.* Kausik, 1939 *a*), for, the *filiform apparatus* becomes somewhat distorted in appearance (Fig. 16) soon after the entry of the pollen tube, while the basal part of the synergid still remains more or less intact.

During fertilization the first male nucleus fuses with the egg (female) nucleus and the second unites with the secondary nucleus formed by the union of the two polar nuclei near the egg apparatus (Fig. 14). At this time there is usually some accumulation of starch grains all round the egg nucleus, and also to some extent near the secondary nucleus.

Development of Endosperm

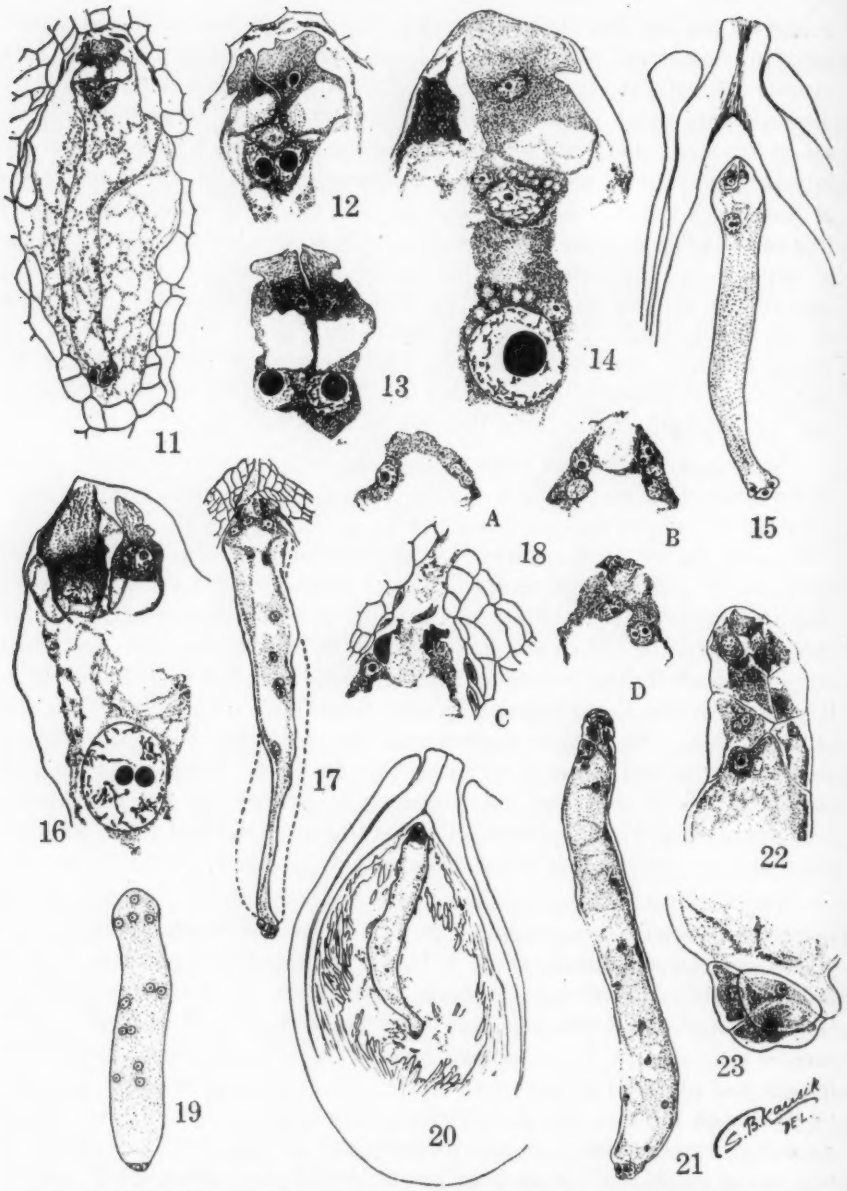
After fertilization the primary endosperm nucleus formed by triple fusion moves down slightly from its former position near the egg apparatus (Fig. 16), and after the lapse of some time gives rise to a number of free endosperm nuclei (Figs. 17 and 18). These nuclei are found mostly in the upper two-thirds of the embryo-sac and are especially concentrated in its micropylar portion where a dense accumulation of cytoplasm is seen. The remains of the pollen tube and a densely staining disorganizing body, which by its position and form appears to be the surviving synergid, are seen at this stage (Fig. 18 *c*), and even later when the development of the endosperm tissue is initiated at the micropylar end of the embryo-sac (Fig. 22).

When the free endosperm nuclei are formed the embryo-sac tends to become slightly zig-zag in appearance (Fig. 17), a tendency which was already foreshadowed even earlier (Fig. 15 ; Pl. X: 1), but which becomes more marked a little later when the endosperm tissue is just formed (Figs. 2, 20, 21 ; Pl. X: 2; Pl. XI: 6–9). It is interesting to compare now the free nuclear stage of the endosperm in Fig. 17 with one of Brough's (1933) figures (*cf.* Fig 70, here reproduced as Fig. 19). The two figures agree closely

except for the fact that the embryo-sac in Fig. 17, especially in its lower part, seems to be narrow, but this is due to shrinkage caused by the fixing fluid in spite of careful treatment. It appears that Brough's figure is drawn slightly diagrammatic so as to give an idea of the general outline of the embryo-sac at this stage, and I say this because of the difficulties I have experienced in getting microtome sections of the embryo-sac with its wall quite intact at this stage; the wall is rather delicate, and there being no tissue around (the surrounding nucellus is almost completely gone in the vicinity of the sac) to support it, nor enough cytoplasm inside to keep it stretched, this part of the embryo-sac becomes easily distorted on account of the action of the fixing fluid. However, by modifying Fig. 17 slightly as shown in dotted outline, which also then corresponds closely to the form of the embryo-sac in dissected preparations (Pl. XI: 6), the resemblance between this and Brough's figure (here Fig. 19) becomes very close.

Subsequent to the free nuclear stage, the inception of the cellular endosperm takes place, and in this it is only the nuclei in the micropylar accumulation of cytoplasm that participate (Figs. 2, 2*a*, 21, 22; Pl. X: 2; Pl. XI: 7–9), while the rest of the embryo-sac still continues to have only free endosperm nuclei and becomes conspicuous as a cœnocytic and definitely worm-like structure (Pl. XI: 9). Further, it is found in entire living preparations at this stage that the early endosperm cells in the micropylar part have their outer free walls bulging out rather conspicuously (Figs. 2, 2*a*; Pl. XI:8) which is presumably due to the pressure on them from inside on account of the rich cell contents. As further development proceeds, the endosperm tissue becomes larger and larger in size with increase in the number of cells, and concomitant with this, the lower cœnocytic portion of the embryo-sac becomes more strikingly worm-like so that it may be referred to hereafter as the *vermiform appendage* of the endosperm (Figs. 3, 26; Pl. X: 3; Pl. XI: 10).

The *vermiform appendage* grows to a length of about 3 to 5 mm., or may even be slightly longer sometimes. Its lower end, where the three antipodals are seen prominently (Figs. 3, 34, 37; Pl. XII: 12), is generally slightly pointed. The wall of the *appendage* appears to be distinctly lamellose (Fig. 33), and within this the cytoplasm is peripheral with a large central vacuole (Fig. 26; Pl. X: 3), which, however, is here and there traversed by strands and plates of cytoplasm (Fig. 33). The presence of the large vacuole, together with the fact that the peripheral cytoplasm may be in some places quite thin, or even almost absent, indicates that the quantity of cytoplasm is here rather insufficient for the large size attained by the *appendage* during its rapid development, and this is also the reason why it collapses frequently in fixed material.



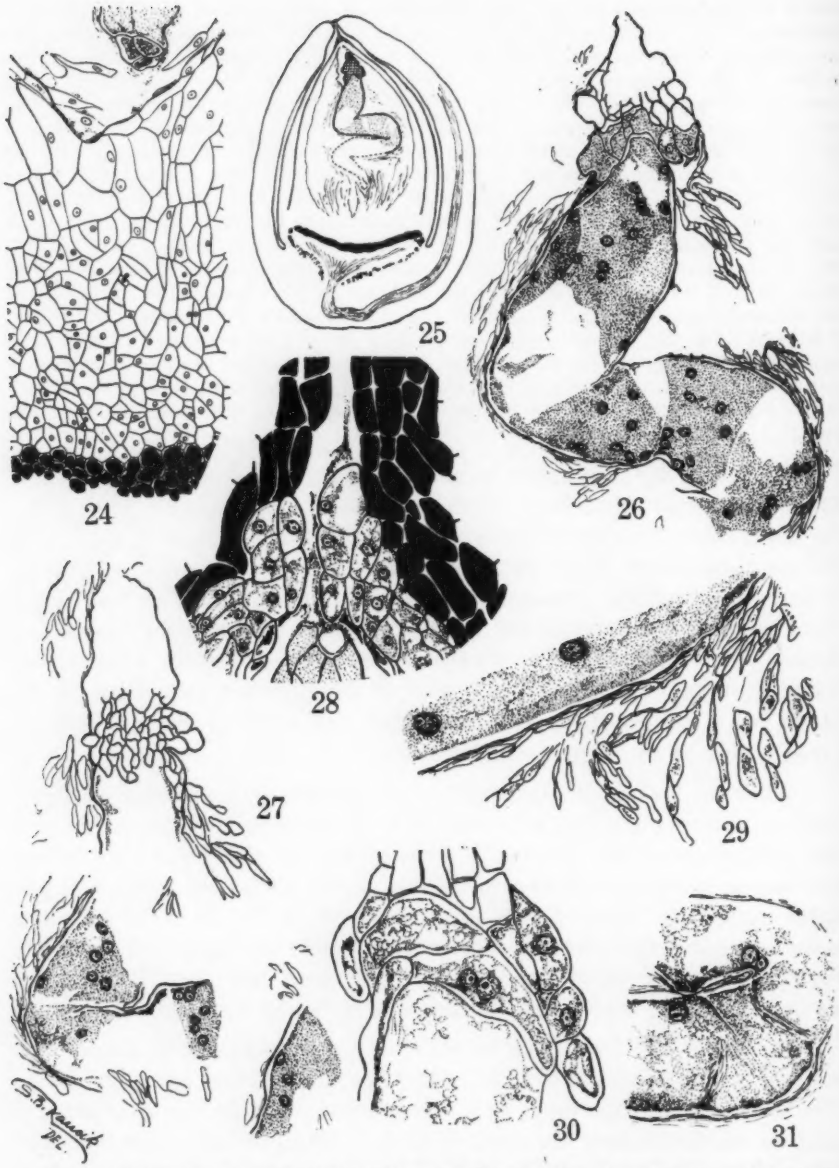
FIGS. 11–23.—Fig. 11. Embryo-sac just before fertilization showing the destruction of the surrounding nucellus and the presence of starch inside the sac (from 3 sections). Fig. 12. Egg apparatus enlarged from Fig. 11. Fig. 13. Egg apparatus from another preparation ; in this

and in the previous figure the synergids show the beaked appearance with the *filiform apparatus* and the egg cell is slightly masked by the synergids. Fig. 14. Fertilization ; note starch grains round the egg nucleus and near the secondary nucleus. Fig. 15. Part of ovule in longitudinal section showing embryo-sac immediately after fertilization ; the sac is slightly curved at the lower end where the three antipodals are seen. Fig. 16. Micropylar part of same enlarged ; the pollen tube is partly masking the egg cell. Fig. 17. Embryo-sac showing free endosperm nuclei which are concentrated in the micropylar part ; the lower part of the sac is shrunk and the dotted outline represents its true form (from 4 sections). Fig. 18 *a*, *b*, *c* and *d*. The four sections used for previous figure drawn separately on a slightly larger scale. The canal formed by the pollen tube in the apical part of the nucellus is seen in Fig. 18 *c*. Fig. 19. Embryo-sac with free endosperm nuclei (from Brough, 1933, drawn here for comparison with Fig. 17). Fig. 20. Longitudinal section of seed showing embryo-sac forming early endosperm cells at the micropylar end, the lower part remaining free nuclear ; note the zig-zag nature of the sac and the destruction of the surrounding nucellus. Fig. 21. Same stage from another section (from 2 sections). Fig. 22. Micropylar part of sac from previous figure enlarged to show early endosperm cells. Fig. 23. Antipodal end of sac from same enlarged to show the three antipodal cells. Figs. 11, 18, 22. ×450 ; Figs. 12, 13, 16, 23. ×900 ; Fig. 14. ×1800 ; Figs. 15, 17. ×200 ; Fig. 19. ×180 (as given by Brough, but here reduced to half) ; Fig. 20. ×80 ; and Fig. 21. ×160.

The *vermiform appendage* invades the large mass of nutritive tissue formed, as already described elsewhere (Brough, 1933; Kausik, 1938 *a*), by the remarkable meristematic activity in the chalazal region of the seed, and the *appendage* seems, by its peculiarly tortuous course, almost to wander, as it were, in search of nutriment for the needs of the future embryo which develops at the micropylar end (Fig. 41). Thus the *appendage* exhibits a definitely haustorial rôle of a very aggressive nature, and finally brings about a thorough and complete destruction of all the adjoining cells (Figs. 26, 29, 41; Pl. X: 3) by dissolving away even their cell walls so that an immense cavity begins to be formed inside the seed.

While the *vermiform appendage* is thus growing and exhibiting a haustorial rôle, certain interesting facts require to be mentioned in connection with the development of the endosperm tissue above. In this tissue a distinction becomes soon apparent between two regions, an upper one with small and rather compactly arranged cells (Figs. 3, 38; Pl. X: 4; Pl. XI: 10), and a lower one with larger and also more or less loosely arranged cells (Fig. 39). Further, a cell of the endosperm tissue may here and there contain two nuclei at first, but becoming uninucleate later by the formation of a wall.

The upper and lower cellular regions in the endosperm tissue are not, however, separated by any clear boundary line; on the other hand, the change from one region to the other is a very gradual one. In this connection it must be mentioned that the writer (Kausik, 1938 *a*) has elsewhere described some " horn-like " cells at the base of the endosperm tissue where it abuts on the proximal end of the *vermiform appendage*. These cells are only some of the very large endosperm cells at the lower region. In some dissected



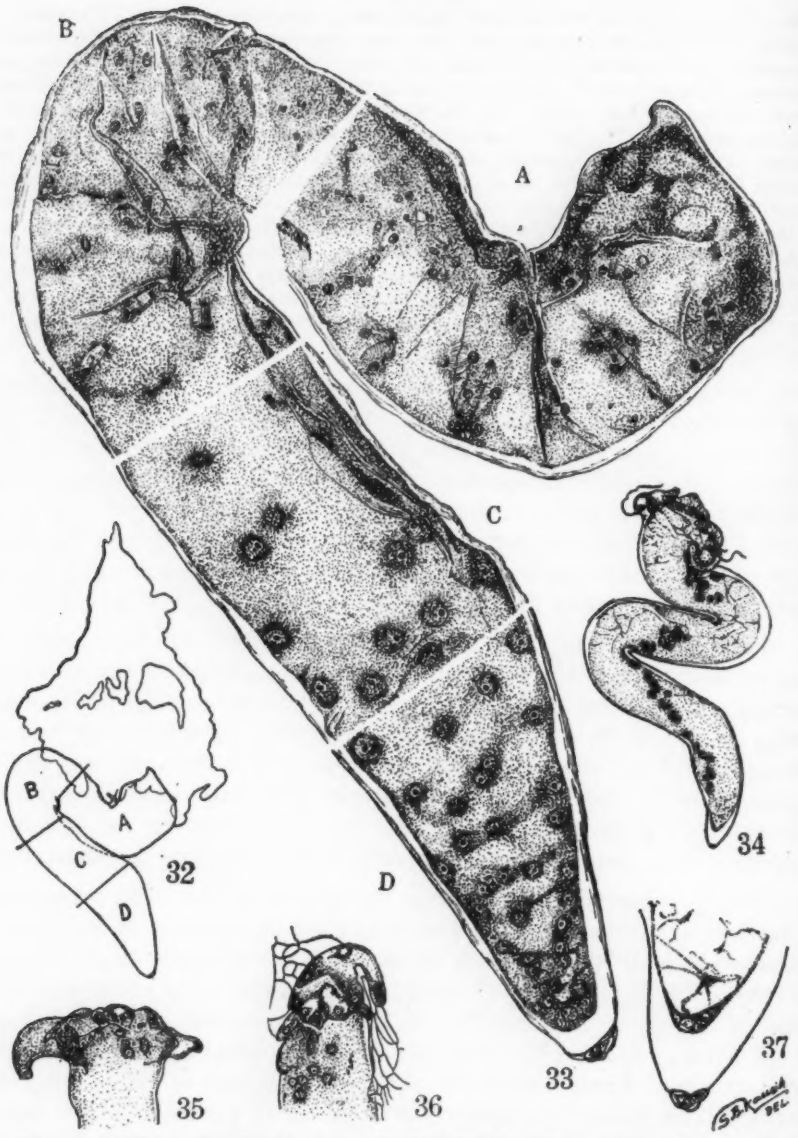
FIGS. 24–31.—Fig. 24. Part of chalazal region of young seed showing the nutritive tissue formed by the chalazal meristematic zone which is above the pad of tannin cells (shown black); the embryo-sac in Fig. 21 is drawn from this preparation; the antipodal end with the antipodal

cells is seen invading the nutritive tissue which forms a shallow pocket. Fig. 25. Longitudinal section of older seed showing embryo-sac with upper endosperm tissue and the lower *vermiform appendage* ; the dotted line indicates that these parts of the *appendage* shown here are taken from two additional sections, while the continuous line shows the endosperm and the *appendage* (part) appearing in one section. Fig. 26. The endosperm and the *appendage* enlarged from previous figure. Fig. 27. Part of *appendage* from the same seed as above but seen in the next section. Fig. 28. Apical glandular tissue of nucellus in young seed showing the canal-like passage formed by the pollen tube ; the basal part of the micropyle and the inner layer of tannin cells of the inner integument (shown black) are also seen. Fig. 29. Part of *appendage* to show the dissolving away of the walls of the surrounding cells of nucellus which thus become separated. Fig. 30. Upper part of *appendage* where it is in contact with the base of the endosperm tissue; note that some of the cells are conspicuous and it is these that become " horn-like " and referred to in the text. Fig. 31. Part of *appendage*, later stage showing the beginning of cross wall formation. Figs. 24, 28, 30. ×450 ; Fig. 25. ×20 ; Figs. 26, 27. ×160 ; Fig. 29. ×400 ; and Fig. 31. ×200.

out preparations these cells were generally about four in number, but varied in other preparations. Similarly, their shape, often strikingly horn-like as described, may also vary to a certain extent (Figs. 34–36). Further, they may not develop prominently at all times, but may be even completely absent in some cases (Figs. 3, 33). When present, however, they are rather conspicuous being sharply marked off from the other large endosperm cells by their densely staining cytoplasm containing one or more nuclei and also by their curious outlines as shown in Figs. 34–36.

It is probable that the conspicuous appearance of these cells is due to the fact that they are situated just at the junction of the two well-marked regions of the embryo-sac, namely, the upper containing the endosperm tissue and the lower forming the *vermiform appendage*, and thus all absorbed nutriment seems to pass through these cells to the upper micropylar region where the embryo develops. Further, these cells are perhaps also of the nature of what may be termed local haustorial structures for absorbing nutriment from some of the nucellar cells in the immediate neighbourhood.

During subsequent development, the growth of the endosperm tissue is very rapid, particularly in the direction of the chalaza and in the antero-posterior plane of the seed with the result that it becomes large and also flattened (Figs. 6, 8; Pl. XII: 13, 15, 17). This growth in the antero-posterior plane is shared by the nucellus, as also by the other component parts of the ovule and seed, and as the sides of the broadening endosperm tissue begin to extend steadily across on either side, the cells of the nucellus are attacked layer by layer successively so that finally a few peripheral layers of the nucellus alone persist immediately within the seed-coat (Fig. 41 ; Pl. X : 4). The destruction of the nucellus in the antero-posterior plane seems to be hastened up when an intimate contact is established between it and the sides of the endosperm tissue, especially near the base of the latter where



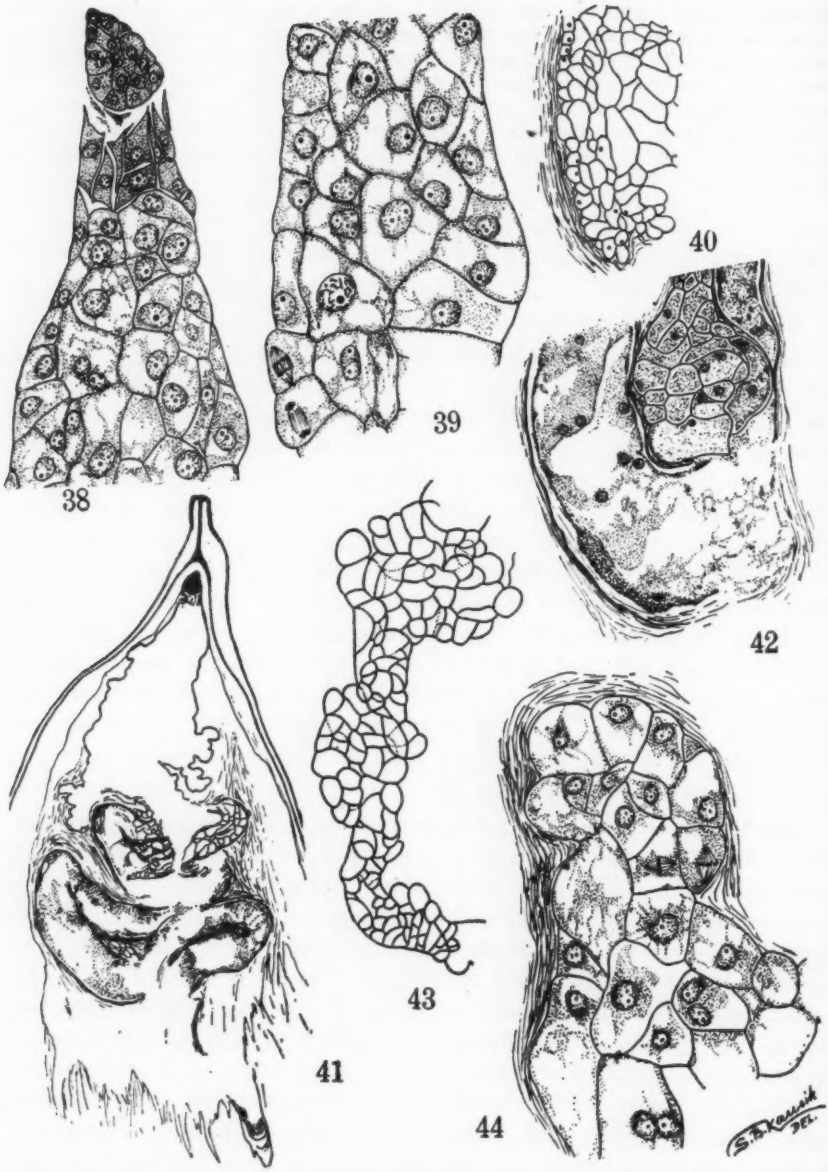
FIGS. 32–37.—Fig. 32. Outline camera lucida drawing of permanent whole mount of endosperm and the *vermiform appendage*. Fig. 33. The *appendage* from previous figure under very high magnification to show nuclear divisions. The spindles were located with the help of a binocular microscope under oil as all parts of the *appendage* cannot appear in one and the same focus. The entire *appendage* was drawn in four parts marked A, B, C and D corresponding to

the regions in Fig. 32 where the sides of the *appendage* at A and C are overlapping. Note the lamellose wall of the *appendage*. Fig. 34. *Vermiform appendage* from another permanent whole mount with free nuclei (this precedes the stage shown in Fig. 33 where the cross partitions are already seen). The upper part of *appendage* shows some " horn-like " cells. Figs. 35 and 36. The different forms of the " horn-like " cells (see text). Fig. 37. The lower end of the *appendage* in Fig. 34 enlarged to show the three prominent antipodal cells; the cytoplasm at the tip is slightly shrunk. Fig. 32. ×50; Fig. 33. ×270; Fig. 34. ×40; Figs. 35, 36. ×80; and Fig. 37. ×200.

some of the peripheral endosperm cells tend to bulge out rather prominently and thus form a number of small invading processes (Figs. 40, 41; Pl. XII: 15, 17). These remind one of the peripheral cells of the foot in the sporophyte of *Anthoceros* (*cf.* Kausik, 1938 *a*).

Now turning our attention again to the *vermiform appendage*, we notice that some very important changes have already been initiated in it at the time when the upper endosperm tissue has become a prominent structure inside the young seed. After the *appendage* has assumed an aggressive haustorial rôle with a steady increase in the number of nuclei, and after a further period of its activity when it dissolves away the cells of the surrounding nutritive tissue (Fig. 41), it becomes septate by the formation of cross partitions here and there along its length (Fig. 4). These partitions seem to arise definitely in relation to the cytoplasmic strands traversing the large vacuole in the *appendage* (Fig. 31). There is, however, no regular sequence in the appearance of these partitions; consequently, the *appendage* forms a number of chambers which are of different sizes (Fig. 4) with the separating walls either transverse or slanting at various angles, especially in slightly later stages (Fig. 33; Pl. XII: 13, 14) when they are also oblique and often curved in an intersecting manner.

The chambers formed in the *appendage* contain a varying number of nuclei (Figs. 4, 33) which subsequently show a further increase in number by regular mitotic divisions followed by wall formation. This mitotic activity seems to proceed gradually and in orderly fashion so that one may see almost all division stages from the most initial in the lower portions of the *appendage* to the most advanced in its upper portions (Fig. 33; Pl. XI: 11). Further, since the dividing nuclei are generally restricted only to such portions in the *appendage* where the cytoplasm is rather dense, cell formation which is, in later stages, a very conspicuous feature, seems to be at first more or less confined only to these portions. The result is that some portions of the *appendage* remain, either completely or almost so, without progressing far in this cell organization. In these portions the cytoplasm becomes rather thin and the nuclei begin to degenerate later. In many preparations these portions were seen sharply marked off from the rest of the *appendage*



FIGS. 38–44.—Fig. 38. Upper part of endosperm tissue ; the young embryo is seen at the top. Fig. 39. Middle part of endosperm tissue ; note that the cells are slightly larger and also

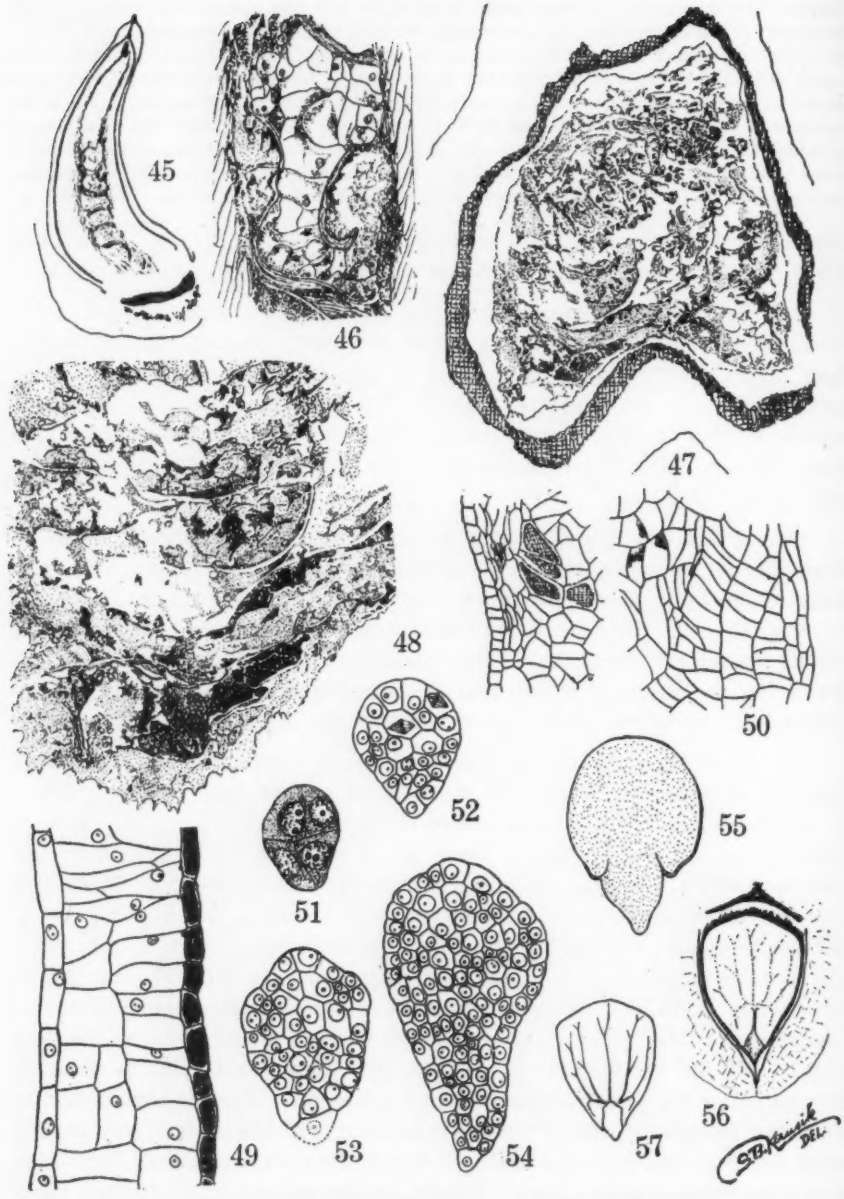
irregular here than in the previous figure : some of the cells also contain two nuclei. Fig. 40. Basal part of endosperm tissue, at a later stage, to show the peripheral invading cells. Fig. 41. Part of young seed in longitudinal section (outer integument is not shown) to show the endosperm tissue and the coils of the *appendage in situ* (from a few sections) ; note the cells in the *appendage* in the upper part ; the embryo is shown black. Fig. 42. A group of cells in the *appendage* formed following cross wall formation. Fig. 43. A much later stage of *appendage* showing the groups of cells becoming very progressive and appearing like clusters (from a living preparation, later killed and mounted). Fig. 44. A group of cells from a microtome section. Figs. 38, 39, 44. ×450 ; Figs. 40, 43. ×80 ; Fig. 41. ×40 ; and Fig. 42. ×200.

where cell formation proceeds rapidly (Figs. 6, 8, 10; Pl. XII: 15, 16, 17). These empty portions begin to shrivel up later in slightly older material.

As cell formation proceeds in the *appendage* as noted above, there result finally groups of small cells, which in several preparations in slightly later stages appear rather like loosely arranged clusters with the peripheral cells bulging out prominently (Figs. 43, 44; Pl. XII: 16). This feature is especially clear in fresh living preparations. In these cell groups a cell may here and there show occasionally two nuclei before finally becoming uninucleate (Fig. 44).

When this cell formation progresses further, the *appendage* begins to show distinct regions with clearly defined cell groups interrupted here and there by portions which remain empty and structureless (Figs. 6-10, 42; Pl. XII: 15, 16). The latter begin subsequently to collapse considerably and shrivel up almost completely in later stages. This is due, as we shall see presently, to certain changes that are initiated in the upper part of the embryo-sac where the endosperm tissue and the embryo are developing.

As already mentioned, the upper endosperm tissue forms a broad and flattened structure on account of pronounced growth both in the direction of the chalaza and also in the antero-posterior plane of the seed. Subsequently, the endosperm fails to keep pace with the rapid growth of the embryo, which also becomes flattened in the antero-posterior plane and soon encroaches upon and increases very much in size at the expense of the endosperm tissue. As further growth of the embryo takes place, the endosperm tissue is pressed more and more from above (Pl. XII: 17) and this in turn causes considerable pressure on the haustorial *vermiform appendage*. Consequently, the coils of the *appendage* become sharply folded together (Figs. 8, 10, 41, 45; Pl. XII: 17, 18) and the empty structureless portions of the *appendage* are almost crushed out of existence. The cell groups in the *appendage* become separated from one another, and as they are very loosely arranged, even the individual cells are frequently detached a little later with continued pressure from above. Finally, as the embryo grows further, there remain only some crushed and disorganized remnants of the *vermiform*



FIGS. 45–57.—Fig. 45. Longitudinal section of seed (cut at right angles to the antero-posterior plane, and so appearing flattened) showing the endosperm tissue in outline and the

coils of the *appendage*. The black area at the base of the seed is the tannin pad. Fig. 46. Part of the *appendage* with a group of cells enlarged from previous figure. Fig. 47. Lower part of seed, slightly tangential, to show the *appendage* at a very late stage when it is undergoing crushing; the coils of the *appendage* can be recognized here and there. The border with cross lines is the tannin layer of the inner integument. Fig. 48. Part of same on a larger scale to show the disorganizing cells of the *appendage*. Fig. 49. Part of inner integument showing the tannin layer (inner epidermis), the palisade-like arrangement of middle layers, and the outer epidermis. Fig. 50. Part of outer integument forming the wing ; note the radial arrangement of cells which are formed by regular tangential divisions and the tannin cells near the inner border. A portion of the tissue of the wing between the inner and outer borders is not shown. Figs. 51–54. Stages in embryo development ; note the pointed lower end and the broader upper end. In Figs. 33 and 54 the embryo is cut at right angles to the antero-posterior plane and so shows the initials of the cotyledons and the stem tip. Fig. 55. Late embryo showing the cotyledons with their basal lobes, *auricles*, and the pointed root-tip (dissected material). Fig. 46. Part of seed, micropyle turned down, showing the old embryo *in situ* ; the endosperm and the *appendage* are completely used up at this stage (diagrammatic). Fig. 57. Embryo shown separately with the *auricles* partly sheathing the radicle (dissected material). Fig. 45. ×20 ; Figs. 46, 50. ×200 ; Figs. 47, 55. ×40 ; Fig. 48. ×100 ; Fig. 49. ×400 ; Fig. 51. ×900 ; Figs. 52–54. ×450 ; and Figs. 56, 57. ×3.

appendage and the endosperm tissue, and both of these together form an almost shapeless mass pressed closely against the tannin-tissue or the *hypostase* found at the base of the seed (Figs. 9, 47; 6). In still later stages, when the seed is passing through the final stages of development, even these crushed remains are more or less completely used up by the embryo, and thus they appear like a brownish highly corroded substance fringing the advancing margin of the embryo. These disorganized cells may be scraped out by means of a needle and examined under the microscope.

Embryogeny and the Structure of the Seed

Some stages in embryo development have already been given elsewhere by the writer (Kausik, 1938 *a*). Brough (1933) states that threre is a gradual change in the shape of the embryo from an earlier oval to a later circular one. This, however, cannot be confirmed according to the observations now made and also previously. On the other hand, the embryo has a definite polarity at all stages; it clearly shows a broad and rounded distal (upper) portion, and a narrower and conical proximal (lower) end (Figs. 51–57).

The development of the embryo is accompanied by increased growth in the antero-posterior plane and thus the embryo becomes flat and disc-like. The cotyledons arise next distally (Fig. 53), and between them lies a central elevated mound of cells forming the stem-apex. These features can be easily seen in longitudinal sections of the seed cut at right angles to the antero-posterior plane. The cotyledons possess each two prominent basal lobe-like prolongations, the *auricles*, which are placed laterally, one on either

side (Figs. 8, 9, 55–57; Pl. XII: 17). According to Lubbock (1892), these are supposed to form a sort of padding tissue to fill up the space which would otherwise be left vacant all round the radicle. While this may be so, it is not clear if any other function may also be ascribed to these structures.

During development some important changes are seen in the structure of the seed. The nucellus is everywhere rapidly encroached upon and its cells become dissolved away by the enlarging embryo-sac, which, though slight in the early stages, becomes a very marked feature later when the endosperm tissue develops rapidly and the haustorial activity of the *vermiform appendage* reaches its maximum. At the same time, the large mass of nutritive tissue formed by the chalazal meristematic zone is also gradually invaded by the *appendage*. In this nutritive tissue an addition of fresh cells takes place as the cells in the immediate neighbourhood of the *appendage* break down (Fig. 24; Pl. X: 2), but after a time the meristematic activity declines and the whole of the nutritive tissue is used up as the seed reaches maturity (Figs. 41, 45). This wholesale destruction of the cells of the nucellus and the nutritive tissue results in the formation of a large cavity inside the developing seed. Within this cavity the endosperm and, later, the embryo are suspended freely.

Conclusions

The synergids seem to be highly specialized with their apex forming the *filiform apparatus* with minute striations. These striations are also figured by Brough (1933; *cf.* Figs. 68, 69), but the general form of the synergids is rather different, and the lateral indentations described here and which give the synergids a distinctly beaked appearance are not shown by him. Beaked synergids have also been previously noted by the writer in *Grevillea Banksii* (Kausik, 1939 *a*) and *Hakea saligna* (Kausik, 1940). As already stated, it is probable that such synergids guide the pollen tubes into the embryo-sac.

A noteworthy feature met with during the development of the seed is the complete destruction of the nucellus and of the nutritive tissue, and this is due to the great increase in the size of the embryo-sac after fertilization when it forms the endosperm tissue and the *vermiform appendage*. The latter is a singularly striking structure met with in *Grevillea robusta* and also in *G. Banksii* (Kausik, 1939 *a*); its presence was also noted in yet another form, *G. Hilliana*. It is, therefore, highly probable that it is a characteristic feature of this genus, although no mention of it is made by Messeri (1928) in the case of *G. macrostachya* where, however, it appears likely that it has

escaped notice, just as Brough (1933) also overlooked its formation in the case of *G. robusta* itself.

Further, it seems that the development of the *vermiform appendage* has some correlation with the formation of an extensive nutritive tissue in the seed. A large nutritive tissue is also present in *Macadamia ternifolia* (Kausik, 1938 *b*), but the *vermiform appendage* is not seen. But here the haustorial lobes formed from the lower end of the embryo-sac after fertilization and containing free endosperm nuclei take up the function of absorbing materials from the nutritive tissue, and are thus clearly of the same nature as the *appendage* in *Grevillea*. It appears, therefore, quite reasonable to suppose that either the *vermiform appendage* or any other similar or corresponding structure is likely to be met with in all cases in the Proteaceæ where a definite chalazal meristematic zone is present and where, consequently, an extensive nutritive tissue is also formed. In this connection it is significant to note that in the case of *Hakea saligna* (Kausik, 1940) there is neither an extensive nutritive tissue (there being no meristematic zone), nor any haustorial structure corresponding to the *appendage* or the embryo-sac lobes.

In the case of *Protea Lepidocarpon* a chalazal meristematic zone is present according to Ballantine (1909), but it is not known if any haustorial structures are formed here. As Ballantine himself says, his work on *Protea* is only a preliminary one, and the details, especially relating to the post-fertilization stages, are so meagre that it appears probable that a detailed study of this form will reveal some interesting features.

Coming to the nature of the *vermiform appendage*, it is definitely to be regarded as a part of the endosperm in the manner of its origin, but is at the same time sharply marked off from it to assume a haustorial rôle. Further, it exhibits two distinct phases in its growth and development. The first phase is characterized by the presence of free endosperm nuclei which steadily increase in number as the *appendage* assumes a more and more strikingly worm-like appearance. An examination of a number of living preparations revealed the fact that the cytoplasm in the *appendage* exhibits a steady and gentle streaming movement in which the nuclei are also slowly carried along. This movement becomes less noticeable later on when cross partitions are formed in the *appendage* and finally stops almost completely in still later stages.

After remaining thus for a time as a cœnocytic structure, the *vermiform appendage* enters into the second phase of its development with the formation of cross partitions in it. Subsequently cell formation begins and, as this

becomes more and more progressive, the *appendage* forms distinct groups of cells. These cell groups in the *appendage* constitute what may be termed a secondary endosperm tissue as contrasted with the primary tissue formed soon after fertilization in the micropylar part of the embryo-sac. In later stages both the first formed endosperm tissue and the secondary tissue formed in the *appendage* are rapidly encroached upon by the embryo and are finally completely used up in the mature seed. Thus the second phase in the development of the *vermiform appendage* comes to a close.

Summary

This paper is a detailed account of the structure and development of the endosperm and the *vermiform appendage*.

After fertilization some free endosperm nuclei are formed, and these are distributed in the upper two-thirds of the embryo-sac, with a strong accumulation at the micropylar end. The organization of cellular endosperm begins a little later, and in this only the nuclei in the micropylar end participate. The rest of the embryo-sac becomes zig-zag in its form and develops into a cœnocytic haustorial structure with a strikingly worm-like appearance. This is designated the *vermiform appendage* which was discovered by the writer. The antipodal cells are seen persisting at this stage and also later.

The *vermiform appendage* shows two distinct phases in its growth and development; the first phase is characterized by the cœnocytic nature of the *appendage* with free endosperm nuclei, and the second by the formation of cross partitions followed later by cell formation to give rise to a secondary endosperm tissue. In mature seeds both the *appendage* and the endosperm tissue are completely used up by the embryo.

The nature and origin of the *vermiform appendage* are discussed in the paper.

In conclusion, I wish to express my sincere thanks to Prof. B. Sahni, of the University of Lucknow, and Dr. P. Maheshwari, of the Dacca University, for kindly suggesting this work and for giving many valuable suggestions and timely advice. I am also grateful to them for the many kind courtesies extended to me during the course of this investigation. Further, I am also thankful to Prof. M. A. Sampathkumaran, Head of the Department of Botany, University of Mysore, for his keen interest in this work and for helpful criticism and advice given to me at various stages.

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EXPLANATION OF PLATES

Plate X

1. Part of longitudinal section of ovule showing upper half of embryo-sac and the surrounding tissues (Fig. 13 is from this section). ×430.
2. Longitudinal section of seed with the embryo-sac showing the early endosperm tissue and the lower free nuclei ; the antipodals are seen as a dark mass. Note the nutritive tissue below the antipodal end of the sac (Figs. 23 and 24 are from this section). ×104.
3. Part of longitudinal section of seed showing endosperm tissue and the major part of the *appendage* ; note the large cavity formed inside the seed (Fig. 26 is from this section). ×104.
4. Later stage showing only the upper endosperm tissue ; the young embryo is also seen here. ×130.

Plate XI

5. Part of longitudinal section of seed showing one of the cell groups of the *appendage* (Fig. 42 is from this section). ×90.
6. Embryo-sac immediately after fertilization showing free endosperm nuclei and the fertilized egg cell (E.C.) ; note the slight curvature of the lower end of the embryo-sac. ×84.
7. Slightly later stage with early endosperm cells at micropylar end, while the lower part of the sac becomes zig-zag. ×84.
8. Same as above, but a little later ; note that the outer walls of some of the endosperm cells are bulging. ×84.
9. A still later stage of the embryo-sac showing the worm-like appearance of the lower part. ×80.
10. A much later stage than before showing the large mass of endosperm and the tubular *appendage* ; the tip of the *appendage* is not in proper focus. ×25.
11. *Vermiform appendage* showing the formation of cross walls and the nuclear divisions; many of the spindles are not seen in focus (Fig. 33 is from this preparation). ×100.

PLATE XII

12. The lower end of the *appendage* from another preparation showing the three prominent antipodal cells (Fig. 37 is from this preparation). ×220.

13. Endosperm and the *appendage* at a later stage showing the formation of cells in the *appendage*; the embryo is seen as a dark body at the top. ×20.

14. Part of the *appendage* (marked in 13) enlarged to show the cells. ×65.

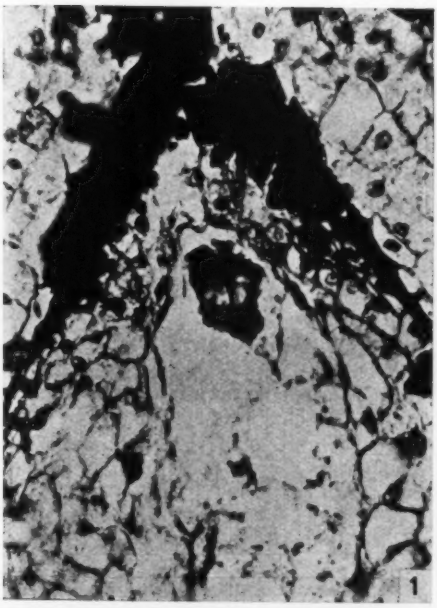
15. A later stage than the one shown in 13, showing the groups of cells and the empty portions of the *appendage* ; the embryo is seen here also. ×15.

16. Part of *appendage* only at a still later stage to show that the groups of cells appear like loose clusters. ×35.

17. Endosperm mass at a very late stage showing the *appendage* folded up and becoming pressed from above ; the dark mass at the top is the embryo showing the cotyledon with the basal lobes on either side of the radicle (directed upwards) ; note the projecting cells of the endosperm at the base for invading the surrounding cells. ×15.

18. The *appendage* at a very late stage with the loops sharply folded and portions shrivelling up.

(*Note*.—Photographs 1–5 are from microtome sections, and the rest are from entire preparations dissected out of the seeds ; of these, 6, 7, 9, 10 and 13–18 are photographs of fresh living material, while 8, 11 and 12 are from killed and stained permanent whole mounts.)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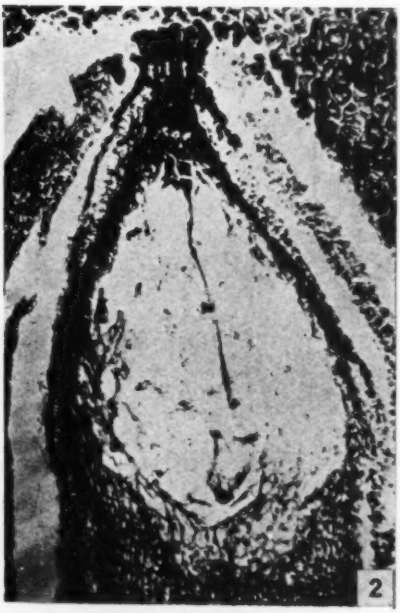
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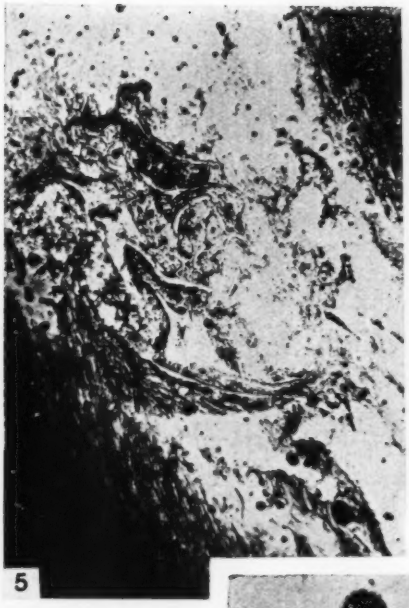
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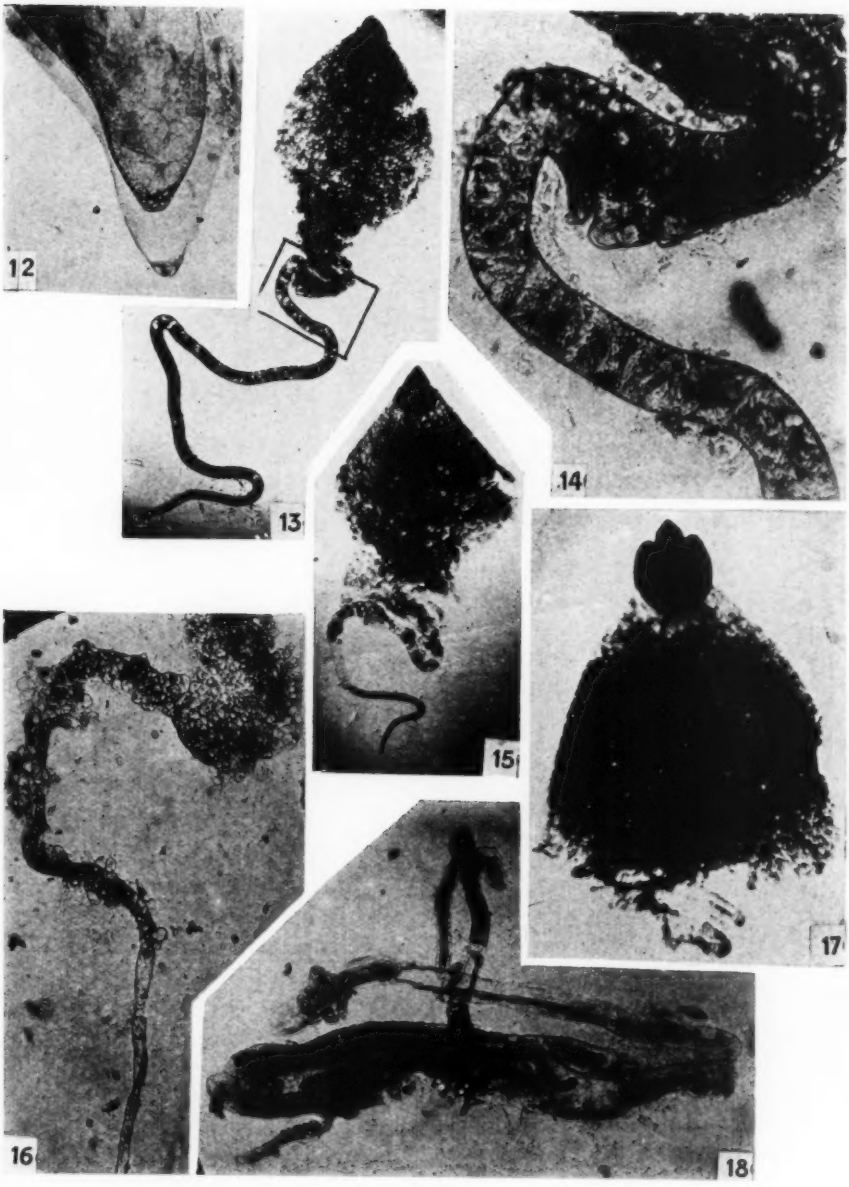
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THE BEARING OF CYTOLOGICAL STUDIES ON THE PHYLOGENY OF THE MARCHANTIALES

By T. S. Mahabalé

(*Department of Biology, Gujarat College, Ahmedabad*)

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Introduction

One of the chief aims of the "*Natural System*" of classification of plants, first propounded by Antoine Laurent de Jussieu in 1789 and adopted subsequently by all the botanists, is to arrange various groups of plants in an order indicative of their relationship or affinities. In building up our phylogenetic schemes in such a system we have to use all the possible data collected from various sources and view them in a proper perspective. Up to the end of the last century such data were forthcoming mainly from the morphological studies; but since the beginning of the present century the science of cytology has opened up a new line of enquiry the findings of which have helped much to clear up some tangles in the mass of morphological evidence at our disposal. The greater part of this evidence pertains to the Angiosperms and that is but natural. The lower plants, however, have not been neglected and the pertinent data gathered in this field though not so vast as in the case of Angiosperms are likely to prove useful in our phylogenetic speculations. It is proposed to review here briefly the evidence obtained from the cytological studies on the liverworts and to indicate its bearing on the phylogeny of the Marchantiales.

The researches on the cytology of the Marchantiales go as far back as Koch (1890–91) and Schottländer (1892) but the most important advances in our knowledge of the cytology of this group are of recent origin. They are mainly due to the work of Allen (1917, 1924, 1925, 1926, 1937), Showalter (1921, 1923, 1928), Haupt (1932, 1933), Heitz (1927, 1928), Lorbeer (1927, 1930, 1934), Höfer (1932), Siler (1934), Wolcott (1939) and others. It is to these authors that we owe much of our knowledge of the chromosomes in the principal genera of the liverworts, though of course, many genera still remain unworked. About 235 genera comprise this group and our cytological knowledge extends to about 50 of them. Apparently we have gained but little insight in the cytology of this group. Similar investigations on the cytology of the Indian forms have been made by workers like

Kashyap and Pande (1922), Pande (1924, 1932, 1933, 1934, 1936), Mehra (1938), Mehra and Mehra (1939), Chavan (1937 *b*), Srinivasan (1939, 1940), Mahabalé and Gorji (1941), but looking to the richness of the liverwort flora in this country a large portion of this field remains to be covered. About 525 species of Indian liverworts have been described by Stephani (1898–1925) to which Kashyap (1929, 1932) and Chopra (1932) added about 50 more; some more forms have been added by Gola (1914), Khanna (1929, 1932 1936, 1937), Verdoorn (1931, 1932 *a*), Chavan (1937 *a*) and others, while our knowledge of their cytology does not extend beyond a dozen forms. Fragmentary as our knowledge is regarding the cytology of the liverworts an attempt is made here to apply it, more in the way of offering some comments on the existing phylogenetic speculations, than for making any original observations.

The Data and Their Analysis

For the sake of convenience the data available have been grouped under the following four headings:—

1. Data pertaining to sex chromosomes,
2. Data pertaining to hybridization,
3. Data pertaining to polyploidy and gene-mutations,
4. Data pertaining to the morphology and number of chromosomes in different genera and species.

1. *The sex chromosomes in the Hepaticeæ.*—The first discovery of the sex chromosomes in plants was made by Allen (1917) in 1917 in *Sphærocarpus Donnellii* and led the way to similar investigation in other diœcious species of liverworts. They have been reported to be present in genera like *Pellia*, *Riccia*, *Pallavicinia*, *Riccardia*, *Lunularia*, *Tesselina*, *Makinoa*, etc. in all about 22 species. But there is some confusion yet regarding their occurrence in species like *Riccardia pinguis* and *Riccia Courtsii* due to faulty nomenclature as has been pointed out by Allen (1935, p. 275). The mechanism so far discovered is of the XY-type differing in detail only in genera like *Lunularia* and *Frullania*. Heteropycnosis was observed in some of them, *e.g.*, in *Pellia Neesiana* by Showalter or in *Hookeria lucens* by Heitz but not in all. We have not yet been able to observe the occurrence of sex chromosomes in many other genera of the liverworts. There is not much difficulty about the diœcious species. The real difficulty is about the hermaphrodite ones. The issue before us is to know how these hermaphrodite forms have come into existence. Have they descended from the diploid races of the originally haploid diœcious parents as Heitz and Allen (1932,

p. 107) suggest, or is the sex metabolic in them as Correns (1928) believes? We are not yet in the possession of any definite answer to this question.

2. *Hybridization.*—Another line of cytological enquiry takes us back to the work of Marchal (1906–1911), Weittstein (1924–1930), Schratz (1924) and others on the hybrid races of mosses. These investigations have been successfully followed by Allen (1924, 1925, 1926, 1937), Burgeff (1930), Haupt (1932, 1933) and others who have worked on the Hepaticeæ. It is well known that the crosses in liverworts under natural conditions are very rare. But since many of the species live in clones, interspecific hybridity may have occurred in them as is known to exist in some species of *Commelina* leading mostly a clonal life. For example, it has been found by Deodikar* that in India there exists a hybrid *Commelina* in nature formed by a cross between *Commelina nudiflora* and *Commelina benghalensis*. There is a common belief among the plant cytologists that many of the species of higher plants to-day have arisen as a result of hybridization among related species of plants. It is well known that many species of garden plants like *Iris*, *Viola*, *Tulipa* and others owe their origin to hybridity. This point of view has been stressed with regard to this and other groups of plants by such eminent investigators as Allen (1935), Tischler (1928, 1935) and Anderson (1937).

3. *Polyploidy and gene-mutations.*—One of the most interesting phenomena from the evolutionary point of view is the phenomenon of polyploidy. Sometimes the whole set of chromosomes forming a basic complex undergoes doubling or trebling and gives rise to polyploids of higher valence. These may be auto- or allo-polyploids and are found in many plants like *Rosa*, *Crepis*, *Chrysanthemum*, *Solanum*, *Primula*, *Carex*, *Agave*, etc. They occur more frequently in plants than in animals. *Apropos* the flowering plants Anderson (1937, p. 343) says: "It is already safe to say that at least half the species and varieties of flowering plants belong to polyploid series (Müntzing, 1936)." But in the lower plants like the Hepaticeæ, as has been remarked by Mehra (1938, p. 6), they are rather rare. They have, however, been recorded in *Pellia epiphylla*, *Pellia borealis*, *Androcryphia confluence*, *Marchantia polymorpha*, *Dumortiera nepalensis*, *Sphærocarpus Donnellii* var. *bivalens*, *Aneura multifida*, *Aneura sinuata*, *Calobryum Neesiana*, *Calobryum trichomanis*, *Riccia Gougetiana*, *Riccia Donnellii*, etc. Whether these are auto- or allo-polyploids, it is difficult to say from the mere numbers of

* I owe this observation to an unpublished work of Mr. G. B. Deodikar working on the genus *Commelina* in India under the guidance of Prof. L. S. S. Kumar of the College of Agriculture, Poona.

chromosomes. Observations on the gene-mutations, chromosomal translocations and on aneuploidy in the Bryophyta are very few and they are mainly due to the work of Heitz (1927, 1928) and Wolcott* (1939) on *Pellia.*

4. *The chromosomes: Their morphology and numbers.*—What holds good for our knowledge of the chromosomal translocations in the Hepaticeæ, also holds good partly at least for our knowledge of the karyotypes of the different genera and species in the liverworts. Some 27 species have been described karyologically by Heitz (1927, 1928), some by Lorbeer (1930, 1934), a few like *Fegatella*, *Riccardia*, *Pellia* and *Pallavicinia* by Showalter (1921, 1923, 1928) and Wolcott (1939) and some of the Indian representatives of the Codoniaceæ by Mehra (1938). We have, however, to describe them in many more genera and find out the homologous pairs of chromosomes. Much work in this direction on the chromosomal complexes of the Orthopteran insects has been done by McClung (1914) and on the homologies of the karyotypes of Reptiles by Matthey (1931, 1933). Obivously there is a great scope for similar work on the karyotypes of liverworts in India. In these circumstances the only recourse left to us for considering our phylogenetic speculations in the light of the cytological data is to make use of whatever scientific information is available regarding the number of chromosomes found in the various forms of liverworts which have been investigated for this purpose. Such information is available in the lists of chromosomes prepared by Ishikawa (1916), Heitz (1927), and Tischler (1931, 1935–1936). Table I gives an abstract of our knowledge of the chromosome numbers in the various groups of the liverworts.

The following points emerge from the table:—

1. In the Ricciaceæ the basic number of chromosomes seems to be 8 as it occurs in all the genera worked out and in the great majority of the species.
2. In the Marchantiaceæ the basic number seems to be 9 as it occurs in the most of the genera worked out.
3. In the Jungermanniaceæ the basic number seems to be 9 as it occurs in most of the genera studied.
4. The chromosome numbers in the Anthocerotaceæ are quite different from those in the majority of the Marchantiales and their separation, therefore, into a distinct family is justifiable even on cytological grounds.

* See also Wolcott, *Science*, 1940, **91**, 593.

TABLE I

Family	The total no. of genera known	Number of genera worked out	Number of species worked out	Analysis of the chromosome numbers	Probable basic numbers	Aberrant chromosome numbers	Polyploid species
1. Jungermanniaceæ	192	29	40	13 genera with $n = 9$ 6 genera with $n = 8$ 2 genera with $n = 18$ 1 genus with $n = 16$ 1 genus with $n = 10$ 1 genus with $n = 20$ 1 genus with $n = 30$	8, 9, 10	..	5
2. Ricciaceæ ..	3	3	23	11 species with $n = 8$ 4 species with $n = 9$ 1 specie with $n = 10$ 1 specie with $n = 16$ 1 specie with $n = 18$ 2 species with $n = 4$	8, 9, 10	..	2
3. Corsiniaceæ ..	3	1	1	1 specie with $n = 16$	8	..	..
4. Targioniaceæ ..	3	2	2	1 specie with $n = 24$ 1 specie with $n = 9$	8, 9	6	1
5. Sauteriaceæ ..	4	2	4	4 species with $n = 9$	9	..	..
6. Rebouliaceæ ..	6	2	6	1 specie with $n = 9$ 5 species with $n = 16$	8, 9	..	5
7. Marchantiaceæ ..	26	13	38	11 species with $n = 9$ 3 species with $n = 8$ 2 species with $n = 10$ 2 species with $n = 18$ 2 species with $n = 27$	8, 9	11, 17, 25	4
8. Anthocerotaceæ ..	5	3	6	2 genera with $n = 6$ 1 genus with $n = 4$	4, 6	5	..

5. Examples showing aberrations from the usual basic numbers are very few.

6. Polyploidy and aneuploidy are of rare occurrence.

7. Numbers 8 and 9 occur very frequently in many genera and families known to be quite unrelated on other grounds. They may have, therefore, arisen polyphyletically.

8. As most of the genera in the Hepaticeæ have 8, 9 or 10 or the multiples and submultiples of these numbers in their haploid, *i.e.*, gametophytic state, the basic numbers in the Hepaticeæ seem to have reached a great stability as in the Gymnosperms where the great majority of the genera are referrable to four basic numbers 10, 11, 12 and 13.

Phylogeny of the Marchantiales

It is necessary at the outset to remark that this is a much vexed question and there will be many who will not agree with the view that will be propounded here, and perhaps with good reasons. The crux of the whole situation lies in the consideration of the simple forms. Have they given rise to more complex forms in evolution or have they passed through an ordeal in course of time and got themselves reduced to simple forms from some complex ancestors? Many eminent investigators like Bower, Campbell, Schiffner, Müller, Cavers and others favour the former alternative while there are other authors equally eminent like Goebel, Kashyap, Weittstein, Church, Howe, Buch, Meyer, Verdoorn, Evans, Pande who favour the latter alternative. The first view considers that the complex forms of the Marchantiales like *Marchantia*, *Pressia* and *Asterella* are the culminating forms of a progressively advancing series beginning with simple forms like *Riccia*. By far the most elaborate statement of this view is given by Cavers (1911) who has summarised his view-point in the following scheme in *New Phytologist*, Vol. IX, p. 180:—

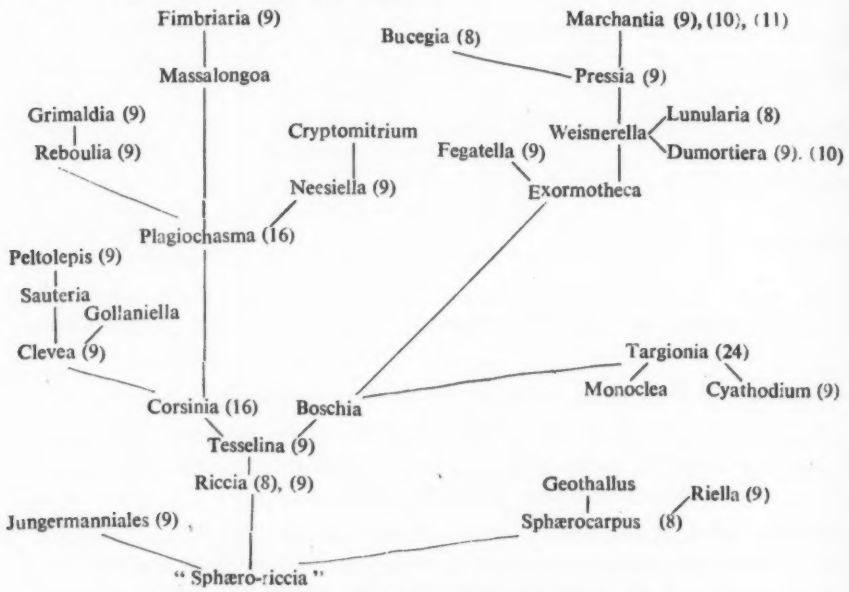

FIG. 1. Phylogeny of the Marchantiales as suggested by Cavers.

The other view, which was brought to the fore by Goebel (1910), was based on his researches on the genus *Monoselenium*, then imported in Germany

by an accident along with some tea plants from Canton, later derived support from Kashyap's masterly discoveries on the peculiar Himalayan genera *Stephensoniella, Aitchisoniella, Sewardiella* and others. The reasons which led Kashyap to accept this view are given in his early papers on the Himalayan liverworts in the *New Phytologist*, Vols. XIV and XV, and have been restated in detail in his presidential address to the Botany Section of the Indian Science Congress, 1919 (see Kashyap, 1919). They are schematically summarised by him in the following diagram (Kashyap, 1914, p. 209) in which as he says (Kashyap, 1932 *a*, p. 2): " *Riccia* is the last term in another series of reduction."

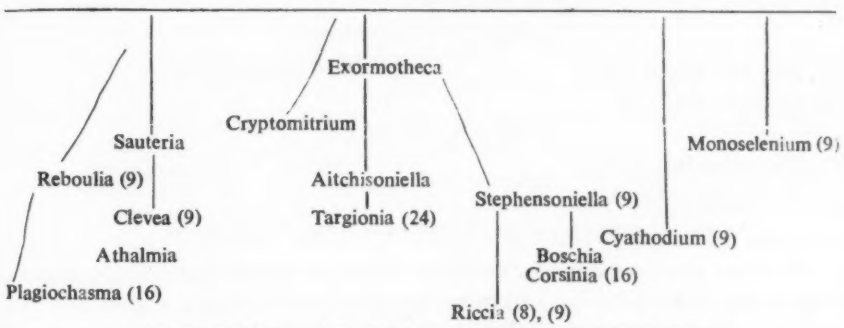

FIG. 2. Phylogeny of the Marchantiales as suggested by Kashyap

Buch, Verdoorn (1932 *b*) and Evans (1939) lend their support to this view and they have elaborated it still further. This modified scheme was first published in the *Annales Bryologici*, Vol. X and later transferred to *Manual of Bryology* by Verdoorn (1932 *b*). It has been published again by Evans (1939) in *Botanical Review*, Vol. V, p. 94. It is as follows:—

Order 1. Jungermanniales.
Order 2. Marchantiales.
Order 3. Sphærocarpales.
Order 4. Anthocerotales.

Order 2. MARCHANTIALES

Family 1. Marchantiaceæ

1. *Marchantia* L. (9), rarely (10)
2. *Marchantiopsis* Ch. and Douin
3. *Pressia* Corda (9)
4. *Boucegia* Radian (8)
5. *Conocephalum* Web. (9)
6. *Wiesnerella* Schiffn
7. *Lunularia* Adans. (8)
8. *Exormotheca* Mitt.
9. *Corbierella* Douin and Trabut
10. *Stephensoniella* Kashyap (9)
11. *Dumortiera* Nees (9), (10)
12. *Monoselenium* Griff. (9)

Family 2. Rebouliaceæ

14. *Reboulia* Raddi (9)
15. *Mannia* Opiz
16. *Asterella* Beauv. (9)
17. *Cryptomitrium* Aust.
18. *Massalongoa* Steph.
19. *Plagiochasma* Lehm. and Lind. (16)

Family 3. Sauteriaceæ

20. *Peltolepis* Lindb. (9)
21. *Sauteria* Nees
22. *Sauchia* Kashyap
23. *Clevea* Lindb. (9)

Family 4. *Targioniaceæ*

24. *Targionia* L. (24)
25. *Aitchisoniella* Kashyap
26. *Cyathodium* Kunze (9)

Family 5. Corsiniaceæ

27. *Corsinia* Raddi (16)
28. *Cronisia* Burkeley
29. *Funicularia* Trevis.

Family 6. Ricciaceæ

30. *Oxymitra* Bisch. (9)
31. *Ricciocarpus* Corda (4)
32. *Riccia* L. (8), rarely (9)

FIG. 3. Phylogeny of the Marchantiales as suggested by Evans.

We shall now consider whether either of these schemes gains any support from recent cytological investigations in this field. In this connection, I have to invite the attention of the reader to the number of chromosomes placed in brackets against the name of each genus, wherever they were available, in all the three schemes of classification. It will be seen that the numbers 8 and 9 occur repeatedly along many lines in genera known to be quite unrelated. This being so we cannot speak of wholesale reduction or wholesale progression along any particular line suggested by the scheme of Cavers or Kashyap. Reduction as well as progression seem to be responsible for what the species are to-day. The question arises which one of the three probable basic numbers 8, 9 and 10 represents the number of chromosomes in the basic complex of the progenitors of the Marchantiales—let us say Pro-Marchantiales. It is perhaps easy to understand the sporadic occurrence of the number 10 in the basic complex of some genera like *Marchantia* or *Dumortiera*. If one of the chromosomes in a basic set of 9 chromosomes of a genus had undergone fragmentation, it might have added one more element to the basic complex, as has been found to be the case in the genom of *Crepis sibirica* by Swezy* (1935, p. 383). Our choice of the basic number of chromosomes in the Pro-Marchantialean complex,

* See also Swezy, *Cytologia, Fujii Jub. Vol.*, 1937, **1**, 149–55.

therefore, should be between 8 and 9. If it be supposed that the basic complex of the Pro-Marchantiales consisted of 8 chromosomes, then we can say that there has been an advance from a series consisting of 8 chromosomes to one having 9 chromosomes. Let us see how far the suggestion is borne out by the scheme of Cavers based on the progression hypothesis. If the number of chromosomes alone were the criterion to judge the advance of a genus, then we would be obliged to suppose that *Bucegia* is not more advanced than *Marchantia* or that *Grimaldia* and *Fimbriaria* are more reduced than *Plagiochasma* which is not consistent with the morphological evidence on which Cavers's scheme is based. The deduction of a complex group like that of *Plagiochasma* from that of a comparatively simple genus like *Corsinia* is logical but the deduction of the chromosomal complex of *Corsinia* from that of *Tesselina* does not follow logically as the latter genus has the basic number 9 and the former has 8. In the same way it is difficult to derive a complex of 9 chromosomes in *Clevea* from that of *Corsinia* with 16 chromosomes, unless of course we suppose that the chromosomal complex of *Corsinia* underwent reduction and then progression. The suggested relationship between *Neesiella* and *Plagiochasma*, the latter with 16 and the former with 9 chromosomes, or that between *Cyathodium* with 9 chromosomes and *Targionia* with 24 chromosomes is likewise inexplicable on the basis of progression hypothesis. These are, therefore, some of the difficulties to be solved by the supporters of the hypothesis that a genus like *Riccia* or what Cavers calls " *Sphæro-riccia* " has given rise to se veral advanced genera of the Marchantiales. Anyway the verdict of the cytological evidence as it stands to-day is not unequivocal on the progression hypothesis.

We may next consider the other two schemes of classification stressing the reduction hypothesis as a factor in the evolution of the Marchantiales. Kashyap's scheme suggests that a form like *Riccia* has been derived from the level of *Marchantia* by a process of reduction. It will be seen from the scheme given on page 147 (Fig. 2) that there are no discrepancies in this scheme along any particular line undergoing reduction. If we consider that the chromosomal complex at the level of *Marchantia* consisted of 9 chromosomes and in course of time got reduced to 8, and occasionally underwent diploidy as for example in *Corsinia* or *Plagiochasma*, then there is little difficulty in interpreting this scheme on the hypothesis of reduction.

The other scheme proposed by Verdoorn, Buch and Evans may now be considered. As regards the arrangement of the genera in this scheme Evans (1939, p. 89) says: " The families in this scheme so far as possible have been

arranged in phylogenetic sequence. This sequence represents a compromise between Verdoorn's and Buch's earlier classification, except that the Anthocerotales are not separated as a sub-class from the rest of the Hepaticeæ." He further states that " in families containing more than one genus the attempt has been made to place genera with primitive characters at the beginning and genera with advanced characters at the close." A careful consideration of the chromosome numbers, so far known, written against several genera in the brackets in this scheme (Fig. 3) will show that there has been a general tendency towards reduction in the whole plexus of the Marchantiales. This tendency seems to have manifested itself in different families independently, so that in each one of them there are certain genera with 8 chromosomes. Thus in the Marchantiaceæ *Bucegia*, in the Rebouliaceæ *Plagiochasma*, in the Targioniaceæ *Targionia* and in the Corsiniaceæ *Corsinia* have 8 chromosomes in their basic complex. When the level of the Ricciaceæ has been reached the tendency becomes more uniform as is evident from the fact that in *Oxymitra* as well as in the great majority of the species of *Riccia* the number of chromosomes in the basic complex is 8. But so long as many genera of the liverworts remain uninvestigated it is difficult to say how far this analysis on cytological grounds alone contains any grain of truth. Provisionally it may be said, however, that cytologically there seems to be some justification for a view which considers that reduction has played an important part in the phylogenetic story of the Marchantiales. At the same time there is no denial of the fact that some progress has been made in some genera like *Fimbriaria* or *Plagiochasma* due to polyploidy and similar phenomena.

But in our attempt to correlate morphological with the cytological data so far known, with a view to seeking support for the validity of any of the phylogenetic schemes mentioned above, we must not lose sight of the fact that the main cytological observation brought in evidence, either for support or criticism of a scheme in the aforesaid discussion is the difference of only one chromosome between the two chromosomal garnitures, one of 8 and the other consisting of 9 elements. It may well seem doubtful whether this cytological observation could be adequate scientific evidence to prove or disprove the validity of any of these hypotheses.

Can we put any other interpretation on the same existing cytological data and press them into the service of reconciling these conflicting phylogenetic theories based on morphological investigations? Instead of regarding either the 8 or the 9 chromosomal complex as the primitive one, would it not be more plausible to postulate a chromosomal complex of 17 complements as the parental or ancestral garniture from which both the existing

complexes with the reduced numbers 8 and 9 have been descended? Why not say that the chromosomal complex of the Pro-Marchantiales contained both 8 and 9 chromosomes in their basic complex, as we find for example in the Rosaceæ ? The great majority of the Rosaceæ has got 9 chromosomes in its basic complex but the sub-family Pomoideæ has only 8. We explain the occurrence of two basic numbers in this family by supposing that both of them were present in the chromosomal complex of the ancestors of the Rosaceæ from which the modern Rosaceæ with the small deterrent group of the Pomoideæ took their origin. The argument *pari passu* can be applied to the two basic numbers found in the living Marchantiales. Instead of saying that the Marchantiaceæ with 9 series got reduced to Ricciaceæ with a series of 8 chromosomes or progressed *vice versa*, we should rather say that both these groups, the 8 and the 9 series, came into existence independently of each other from the basic complex of the Pro-Marchantiales consisting of 17 chromosomes.

As a matter of fact a view somewhat similar to the one propounded here, suggesting a polyphyletic origin of the Marchantiaceæ and the Ricciaceæ, has been maintained by Meyer (1914, 1931), though of course on absolutely different grounds. Meyer's view is based on ontogeny. In genera like *Marchantia* or *Fegatella* the embryo is of the *filamentous type* whereas in the genera *Riccia* and *Corsinia* it is of the *quadrant type*. On this basis he divides the whole group of the Marchantiales into two classes : those in which the embryo is of the *filamentous type* and those in which it is of the *quadrant type*. From these observations it is argued, that as the embryos of the Ricciaceæ are simpler than the filamentous embryos of the Marchantiaceæ, the progenitors of the Ricciaceæ were perhaps more simpler forms than the modern complicated Marchantiaceæ. Meyer's conclusion stands true to-day though in a different perspective. The cytological analysis clearly suggests a polyphyletic origin for the Ricciaceæ and the Marchantiaceæ. They rather represent end products of two distinct series which have moved along parallel lines but which tend to converge in their evolutionary history in the past.

Considered as a whole the Bryophyta seem to be a very conservative group of plants in the process of evolution, as is evident from the great stability of their basic numbers. It resembles in this respect an equally ancient and conservative group of plants, the Gymnosperms, in which also the known chromosomal complexes are reducible to three or four basic numbers. For example, in the whole of Coniferæ the great majority of the genera of the Taxaceæ and Abietinæ has 12 chromosomes and the Taxodinæ and Cupresseæ have 11. The delicate structure and the flabby parts of

the liverworts are not very well suited for fossilisation, and therefore, their fossil record is bound to be imperfect. The only authentic record of the fossil Marchantiales, so far as I am aware, is the Jurassic genus *Marchantites* of Dr. Scott. Some more forms like *Hepaticites* have also been described by Walton (1925, 1928) from the Carboniferous strata of England. But what happened to them in subsequent epochs we do not know from the fossil record. Our only clues, therefore, regarding the course of their evolution are those from the living forms. And to unravel these we have to follow several lines of investigation before their phylogenetic story can be reconstructed. This brings us back to the point from where we started. After all in building up our phylogenetic schemes of a particular group or groups of plants or animals cytology furnishes us with the data obtained by following only one line of enquiry which in themselves are not so important as in their relation to the data obtained by following the other lines of enquiries like morphology, ecology, geographic distribution, etc.; and our phylogenetic notions, therefore, should be based on the sum-total of all the data available from different sources rather than on this or that factor affecting the course of evolution.

Summary

The paper gives a brief account of the cytological studies on the Hepaticeæ and indicates the bearing of these on the problem of the phylogeny of the Marchantiales. The data available have been analysed and grouped under the following four headings: the sex chromosomes, hybridization, polyploidy and gene mutations, and the morphology and numbers of chromosomes. The information regarding the chromosomal numbers has been tabulated and the probable basic numbers in different families have been indicated. From the table given on page 145 it will be seen that the basic number of chromosomes (gametophytic number, *i.e.*, *n*-number) for the majority of the genera of the Marchantiaceæ and the Jungermanniaceæ seems to be 9, and for the genera of the Ricciaceæ it seems to be 8. In the light of this cytological observation the existing schemes of classification of the Marchantiales have been examined with a view to knowing how far the morphological data and the cytological data are correlated. If it be supposed that the basic complex of the ancestors of the Marchantiales called Pro-Marchantiales in this paper, had 8 chromosomes in their genom and changed to a 9 chromosomal condition later by fragmentation, then there is some justification for the progression hypothesis. On the contrary, if it be imagined that the Pro-Marchantialean complex consisted of 9 chromosomes originally and got reduced to 8, the reduction hypothesis may seem plausible. But when either of these ideas are pressed further and

the number of chromosomes in different genera in the various schemes of classification given by Cavers, Kashyap, Verdoorn and Evans are considered, the verdict of cytology on either of these schemes is not unequivocal, as the main argument brought in the discussion for or against a theory is based on the difference of a single chromosome only; and it may well seem doubtful whether that alone can constitute sufficient proof for or against a scheme.

In these circumstances a third reading of the same cytological data is proposed by the author. It is postulated that the Pro-Marchantialean complex consisted of 17 chromosomes from which the Marchantiaceæ and the Ricciaceæ took their origin independently of each other polyphyletically, by the segregation of the ancestral complex into two series one having 9 and the other having 8 chomosomes. The Rosaceæ have been cited as a parallel example which has a bearing on this point. The great majority of the Rosaceæ has 9 chromosomes in their basic complex (*n*-number), but the small subfamily Pomoideæ have only 8. The presence of two basic numbers in this family is accounted for by supposing that the ancestors of the modern Rosaceæ had 17 chromosomes in their basic complex. The argument *pari passu* may hold good for the Marchantiaceæ and Ricciaceæ also. On this assumption the cytological analysis clearly suggests a polyphyletic origin for the Marchantiaceæ and the Ricciaceæ in the evolutionary history of the Marchantiales.

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CONTRIBUTION TO THE MORPHOLOGY OF *PEDALIUM MUREX* LINN. AND *SESAMUM INDICUM* D.C.

By A. R. Srinivasan, M.Sc.
(*Annamalai University*)

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Contents.—I. Introduction. II. Materials and Methods. III. *Pedalium murex* Linn. : (*a*) Microsporangium development ; (*b*) Megasporogenesis, endosperm and embryo development. IV. *Sesamum indicum* D.C. : (*a*) Megasporogenesis ; (*b*) Endosperm haustorium formation. V. Chromosome Numbers. VI. Summary. VII. Acknowledgement. VIII. Literature Cited.

I. Introduction

The family Pedaliaceæ is very small and seems to have been paid very little attention. Only three genera seem to have been investigated. According to Schnarf (1931), *Sesamum* and *Ceratotheca* were investigated by Balicka-Iwanowska (1899). In both the plants micropylar and chalazal endosperm haustroria were met with. Oliver (1888) worked in the genus *Trapella*, where also he found both types of endosperm haustoria occurring.

Records of chromosome number in the Pedaliaceæ are also few. Suguira (1936) found that the haploid chromosome number of *Sesamum indicum* to be thirteen. Ramanujam (1941) reported the chromosome number of another species of the same genus, *S. prostratum* as sixteen.

Though *Sesamum* has been worked out previously, details of development were not available for the present species. Moreover, the genus *Pedalium* has not been investigated. In the present communication, a detailed description of the morphological features of *Pedalium* is given ; a comparative study of the haustorium formation in the two genera, *Pedalium* and *Sesamum*, is also made.

II. Materials and Methods

Pedalium murex Linn. is a stout-stemmed herb easily recognised by its lobed leaves and pale yellow trumpet-shaped flower and spiny fruits. The leaves are extremely slimy when wetted. Stem is about one foot high,

not much branched. Leaves are opposite and decussate, about 1¼ inches long. Petiole, one inch long, with two black glands near the base. Flowers, solitary and axillary, borne on short pedicels. Corolla, pale yellow; Stamens are four in number, didynamous, with distinctly rounded anther halves. There is a rudimentary, short, fifth stamen present, which bears no anther. Ovary two-loculed with pendulous ovules. Fruits conical, with stout spines.

Sesamum indicum Linn. The plant taken for investigation is the white-seeded variety of the commonly cultivated " Gingel " or Sesame.

Root-tips from plants grown in pots were fixed in Navashin's fluid. The flower buds were also fixed in the same fluid after determining the correct stage for fixation through acetocarmine examination. For embryo-sac and embryo stages, the ovaries were fixed in formalin acetic alcohol. All the materials were dehydrated in alcohol and embedded in paraffin wax. The sections were cut at thicknesses varying from 7 to 16 microns and stained in Haidenhein's iron-alum hæmotoxylin.

III. Pedalium murex Linn.

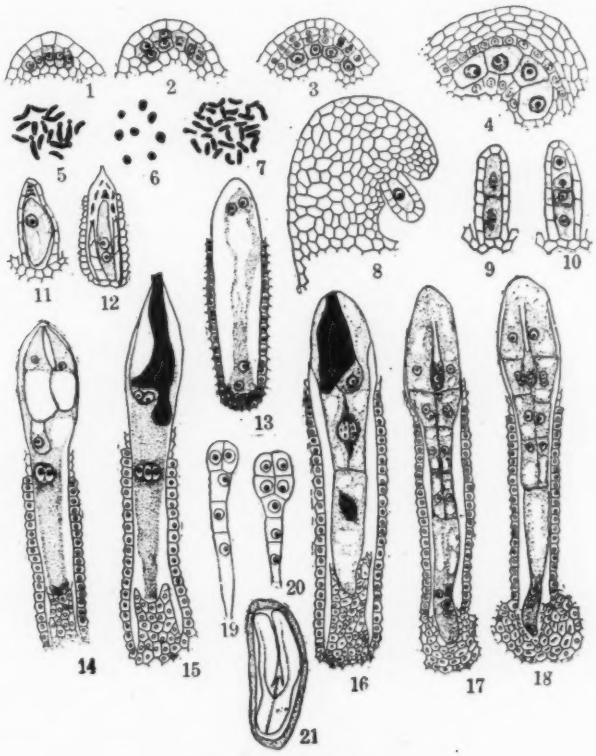
(*a*) *Microsporangium development.*—The archesporium of the anther is differentiated at the four corners of the anther as a hypodermal band consisting of five to six cells (Fig. 1). Such extensive archesporia are not uncommon, one of the latest to be reported being in *Nicotiana glutinosa* (Raghavan and Srinivasan, A. R., 1941 *a*). The archesporial cells divide periclinally giving rise to two layers, the outer forming the primary wall layer and the inner, the primary sporogenous layer (Fig. 2). The primary wall layer undergoes more periclinal divisions giving rise to more wall layers (Fig. 3). The mature anther has got a wall made up of five layers, the innermost of which functions as the tapetum (Fig. 4). The tapetum is uninucleate to begin with, but later, more nuclei are formed by ordinary mitosis and these ultimately fuse. Such a phenomenon has recently been found to be very common and its possible significance has been discussed in detail elsewhere (Raghavan and Srinivasan, A. R., 1941 *b*). There is only a single row of pollen-mother cells indicating that the primary sporogenous cells function directly as the pollen-mother cells without undergoing any divisions. The pollen mother cells are very large compared to the surrounding cells. Their nuclei are also large. In the tapetal cells vacuolation takes place to a great extent and the multi-nucleolate nuclei are relegated to a position near the wall layers.

During meiosis eight bivalents are formed and at first metaphase they lie in the equatorial plate (Fig. 6). They are arranged more or less at

equal distances from each other. Three of the bivalents seem to be somewhat larger than the rest. Probably these are the bivalents organised from the three pairs of chromosomes, which are the largest in the somatic complement.

Meiosis is normal and viable and healthy pollen grains are produced.

(*b*) *Megasporogenesis, endosperm and embryo development.*—The archesporium of the ovule is differentiated in the hypodermal position as a



FIGS. 1–21. *Pedalium murex*

Fig. 1. Archesporium of the anther. **Fig. 2.** Division of the archesporial cells resulting in the primary wall layer outside and the primary sporogenous layer inside. **Fig. 3.** Division of the cells in the primary wall layer. **Fig. 4.** Mature anther showing five wall layers the innermost forming the tapetum. **Figs. 1–4** are of magnification ×750. **Fig. 5.** Somatic metaphase plate in *Pedalium* $2n = 16$. **Fig. 6.** Metaphase I in the same plant. **Fig. 7.** Diploid chromosome complement of *Martynia diandra*, $2n = 32$. **Figs. 5–7** are ×4000. **Figs. 8–15** represent megasporogenesis and fertilisation stages in *Pedalium*. Magnification ×750. Explanation in the text. **Figs. 16–18.** Show stages of endosperm haustorium development in the same plant. **Fig. 16** ×750; **Figs. 17 and 18** ×500. **Figs. 19 and 20.** Embryo stages. ×750. **Fig. 21.** Section through the mature seed. ×20.

single cell which elongates into the megaspore mother cell (Fig. 8). The megaspore-mother cell is enveloped by a single layer of cells, the nucellus. The presence of this short-lived and much reduced layer of mucellus seems to be characteristic of the Sympetaleæ. In the family Rubiaceæ they show a variety in their configuration, which, when studied in correlation with cytological data, was found to be of much use in discussing the phylogeny of the genera in that family (Raghavan and Srinivasan, A. R., 1941 *c*). The nucleus of the megaspore-mother cell undergoes the meiotic division and the dyad is formed (Fig. 9). In the dyad the two daughter nuclei again divide. Their plane of division is in a straight line. Consequent on this division, the linear tetrad is formed (Fig. 10) of which the chalazal megaspore develops into the uni-nucleate embryo-sac. The nucellar layer in the present case still persists intact, while usually it begins to degenerate at this stage (Fig. 11). Even during the bi-nucleate embryo-sac stages, only the micropylar part of the nucellus undergoes degeneration, while the basal portion still persists, though presenting a crushed appearance (Fig. 12). The bi-nucleate embryo-sac is longer and narrower than the uni-nueleate embryo-sac, and the nuclei have not yet migrated to the poles. Soon however they reach the two ends of the embryo-sac and there divide to give rise to the four-nucleate embryo-sac.

The four-nucleate embryo-sac (Fig. 13) is long and considerably broader in size as compared to the bi-nucleate embryo-sac. By the time the four-nucleate embryo-sac is fully formed, the nucellar layer completely degenerates and the innermost layer of the single integument, which thus comes in direct contact with the embryo-sac, is differentiated as the tapetum (Fig. 13). The integumentary tapetum also, like the single-layered nucellus, is characteristic of many sympetalous families. The tapetum in the present case does not surround the whole embryo-sac. In the micropylar region only ordinary cells are met with. During later stages, the tapetum is seen only in the narrow portion of the embryo-sac (Figs. 13-18). In the chalazal end, the tapetum extends far beyond the embryo-sac, thus enclosing some of the chalazal integumental tissue within its limits (Figs. 13–16). The formation of such a ' tapetal jacket ' and a chalazal nutritive tissue, has been met with in *Lobelia* (Kausik, 1935) and in *Limnanthemum cristatum* (Srinivasan, 1941). In the present case the tissue is very prominent and the whole thing degenerates when the chalazal endospern haustorium grows into it (Figs. 16–18).

Thus, the chalazal nutritive tissue assumes the nutritive rôle in two definite periods of its existence. The nutritive material is absorbed from this tissue and transported to the embryo-sac during pre-fertilisation stages by the cells composing the 'tapetal jacket'. In the post-fertilization stages, the

haustorial function is taken up by the chalazal endosperm haustorium. Thus, both during the pre- and the post-fertilisation stages, it has a nutritive function. In this respect, it is more like the nutritive tissue met with in *Lobelia* than that of *Limnanthemum*. For, in the latter, the nutritive rôle of the tissue is restricted to the pre-fertilisation stages, as no endosperm haustoria are formed.

The mature embryo-sac (Fig. 14) is very long. At the micropylar portion it is somewhat broader. Two large synergid cells with basal vacuoles, and a long egg cell constitute the egg-apparatus at the broader micropylar portion of the embryo-sac. The fusion of the polar nuclei takes place even before fertilisation. The fusion product, which is the secondary nucleus, lies in the middle of the embryo-sac. Three antipodals are formed, but are extremely ephemeral (Fig. 14).

During fertilisation, the pollen tube enters through the micropyle into the embryo-sac and hence the process is porogamy. When the massive pollen tube enters the embryo-sac, it crushes the synergids which are on the way. The synergids are broken down and soon degenerate, so that at a later stage, when the gametes fuse with the egg and the secondary nuclei, there is no sign, even of the remnants of the synergids (Fig. 15). The two male gametes are let out from the pollen tube. The male nuclei are spherical. Vermiform and spiral-shaped nuclei are held to be commonest in Angiosperms. Instances of spherical nuclei, however, are not wanting. Weinstein (1926) in *Phaseolus vulgaris*, Madge (1929) in *Viola odorata*, Newman (1934) in *Acacia Baileyana* and Raghavan (1937) in *Cleome Chelidonil* have reported the occurrence of such spherical nuclei. One of the gametes fuses with the secondary nucleus while, the other fuses with the egg-nucleus. As a result of this fusion, the oospore and the endosperm nuclei are formed. All the nuclei are in a resting condition at the time of the fusion. This feature seems to be common not only in Angiosperms, but also in Coniferales (Guillermond, 1933) and in some Cycadales (Lawson, 1926).

The endosperm nucleus is the first to divide. The endosperm is cellular. Fig. 16 shows the three-celled endosperm. In the micropylar portion of the endosperm, wall formation has just been completed. This is revealed by the position of the two daughter nuclei around which cytoplasm is very thick. In the lower cell, the nucleus is in the process of division. From this it is clear that the first division of the endosperm nucleus is transverse and that the micropylar daughter cell is the first to undergo a longitudinal division. The chalazal daughter cell functions as the chalazal haustorium, after its nucleus has undergone two divisions resulting in four

nuclei. This tetra-nucleate haustorial cell has its end in direct contact with the chalazal nutritive tissue (Figs. 16–18). As this haustorial cell increases in length, the chalazal tissue degenerates (Fig. 16) and at later stages the haustorial cell penetrates into the integumental tissue proper, which is outside the limits of the tapetum.

The micropylar cell is responsible for the development of the endosperm. First, it undergoes a longitudinal division (Fig. 16). More transverse divisions follow in each daughter cell to give rise to more tiers of cells (Figs. 17 and 18). Through further divisions the endosperm tissue is formed. Micropylar haustorium is not formed.

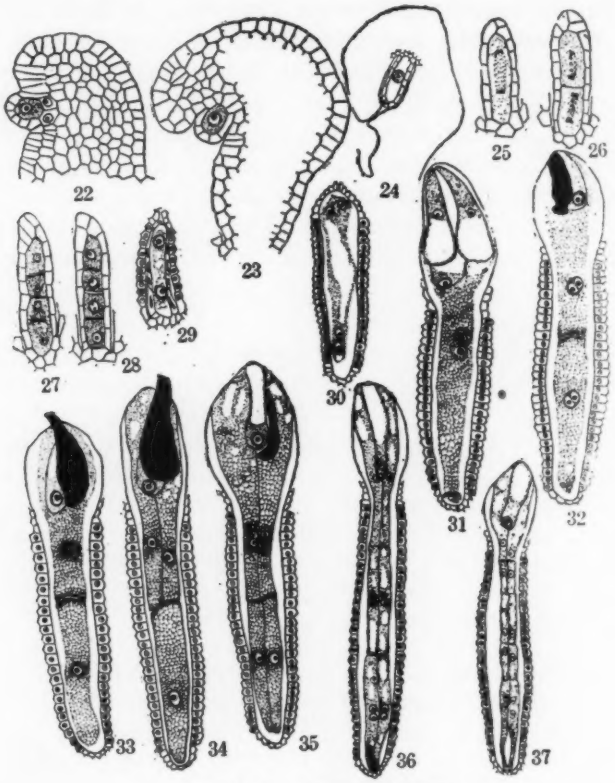
The embryo development conforms to the normal type. Through transverse divisions of the oospore a linear proembryo of four cells is formed, of which, the apical cell undergoes an anticlinal division (Fig. 19). The quadrant and the octants are formed (Fig. 20). The embryo proper is organised from the octant. Through repeated divisions, a spherical mass of cells is formed and during further development, the lobing of the cotyledons takes place.

In the mature seed (Fig. 21) the massive embryo fills up the cavity of the seed. The seed coat consists of a few layers of cells, which are the remnants of the single massive integument.

IV. Sesamum indicum Linn.

(*a*) *Megasporogenesis.*—In *Sesamum indicum*, the development of the female gametophyte takes place in the same manner, as described for *Pedalium*. During early stages the archesporium appears to be multicellular, consisting of two to three cells (Fig. 22). But only one cell develops into the megaspore-mother cell (Figs. 23 and 24). After the usual tetrad and embryo-sac stages, the mature embryo-sac is formed (Figs. 25–31). In the mature embryo-sac, no chalazal nutritive tissue is present (Fig. 31). The cytoplasm is very vacuolate. But for these differences, the details of development of embryo-sac are the same as in the case of *Pedalium*.

(*b*) *Endosperm haustorium formation.*—The essential difference between the two plants taken for the present investigation lies in the manner of development of the endosperm haustorium. The triple fusion nucleus undergoes first a transverse division as a result of which, two daughter cells are formed, one superimposed on the other (Fig. 32). Of these two cells, the micropylar cell undergoes a longitudinal division, as is met with in the case of *Pedalium* (Figs. 33 and 34). After this, the chalazal cell also divides longitudinally giving rise to two cells (Fig. 35). At this stage, the



FIGS. 22–37. *Sesamum indicum*

All the figures except 24, 36 and 37 are of magnification ×750; Fig. 24 ×375 ; Fig. 36 ×500 . Fig. 37. ×375. Figs. 22 and 23. Ovules showing archesporium. Figs. 24 and 25. Megaspore; mother cell stages (division seen in Fig. 25). Fig. 26. Dyad. Fig. 27. Tetrad formation- Fig. 28. Linear tetrad. Fig. 29. Bi-nucleate embryo-sac. Fig. 30. Four-nucleate embryo-sac. Fig. 31. Mature embryo-sac. Figs. 32–37. Show stages of endosperm haustorium formation (explanation in the text).

endosperm is four-celled. The upper two of the four cells develop into the endosperm tissue through repeated transverse divisions. The lower two cells do not divide, but their nuclei divide once and thus they become bi-nucleate, (Figs. 36 and 37). The tips of these cells butt against the integumental tissue at the chalazal end of the embryo-sac. Food material is absorbed from this tissue by the two endosperm cells which thus become haustorial in function. Though the occurrence of micropylar haustorium has been reported in *Sesamum* by Balicka-Iwanowska (1899) in the present case it is not met with. In *Pedalium* also, no micropylar haustorium is formed.

Though chalazal haustorium is found to develop in both the cases, in *Pedalium* it is unicellular and tetranucleate, while in *Sesamum* it is made up of two bi-nucleate cells. Two-celled chalazal haustoria are of common occurrence in Scrophulariaceæ (Krishna Iyengar, 1931; Raghavan and Srinivasan, V. K., 1941). Single-celled multi-nucleate chalazal haustoria, as is met with in the case of *Pedalium*, are also not rare. Krishna Iyengar (1942) has recently recorded the occurrence of three to five nuclei in the single chalazal haustorial cell in *Rehmannia*. Such multi-nucleate haustorium is a common feature in the Labiateæ (Junell, 1937; Narasimhamurthy, 1940).

V. Chromosome Numbers

(*a*) *Pedalium murex* Linn.—(Figs. 5 and 6.) There are sixteen chromosomes in the somatic metaphase plates (Fig. 5). Of these, six seem to be fairly larger than the rest. These large chromosomes, classified on the basis of the nature of their constrictions, fall into three groups, one pair showing satellites, another pair with sub-median constrictions and the remaining one pair exhibiting subterminal constrictions. The rest of the chromosomes are shorter and have subterminal constrictions. The eight bivalents seen in the first meiotic metaphase (Fig. 6) are organised from the eight pairs of chromosomes. The meiotic count confirms that eight is the haploid number of the plant.

(*b*) *Martynia diandra* Glox. (=*M. annua* Linn. Fig. 7).—*Martynia*, though grouped under a different family, is very closely allied to Pedaliaceæ, inasmuch as successful crosses could be effected between it and *Sesamum*. It was however found, that the seeds from this inter-generic cross failed to germinate. The somatic chromosome number of the present species of *Martynia* is determined for the first time to be thirty two. Except a few, all the other chromosomes are generally small (Fig. 7).

VI. Summary

The development of microsporangium in *Pedalium murex* Linn., is of the normal type. The cells of the primary sporogenous layer function directly as the pollen mother cells. The tapetum is multi-nucleate and exhibits nuclear fusion, prior to disintegration.

In *Sesamum* and *Pedalium* the embryo-sac development conforms to the normal type. Chalazal nutritive tissue is met with in *Pedalium*, while it is absent in *Sesamum*.

Endosperm is cellular. The occurrence of chalazal endosperm haustoria is reported in both the genera. No micropylar haustorium was

observed. The chalazal haustorium is single celled and four nucleate in *Pedalium*, while it is made up of two bi-nucleate cells is *Sesamum*.

The diploid chromosome numbers of *Pedalium murex* and *Martynia diandra* have been determined for the first time to be sixteen and thirty-two respectively. In the case of *Pedalium* the number is confirmed from meiotic counts also which show $n = 8$.

VII. Acknowledgement

The work was carried out in the Botanical Laboratory of the Annamalai University. It is a source of sincere pleasure to record my indebtedness to Dr. T. S. Raghavan, M.A., Ph.D (Lond.), F.L.S., Professor and Head of the Department of Botany, under whose guidance the investigation was made.

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TWO NEW SPECIES OF *DIDYMOPSORA* FROM INDIA

By M. J. Thirumalachar

(Department of Botany, Central College, Bangalore)

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(Communicated by Prof. L. Rama Rao)

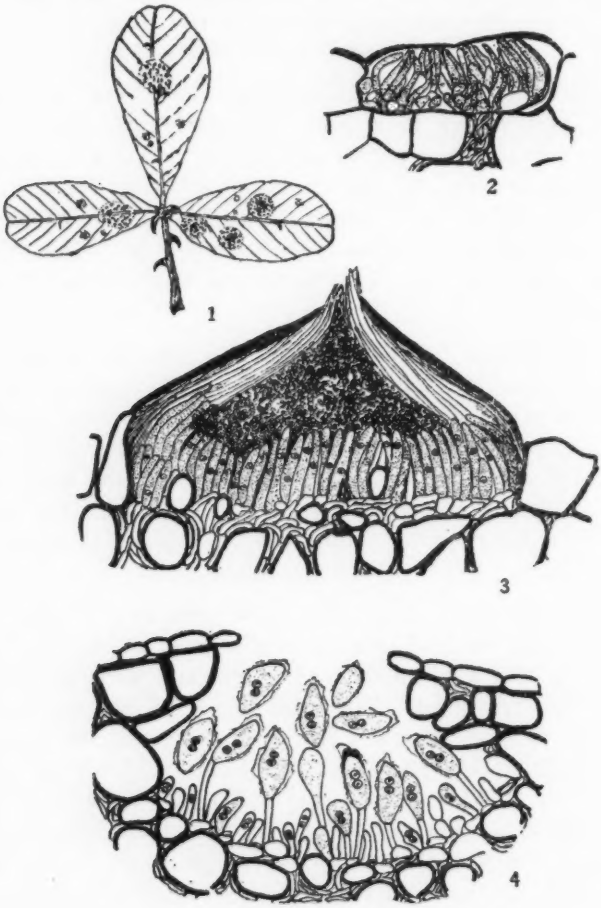
As far back as 1908 the rust on *Toddalia aculeata* Pers. was collected by Petch (1908) who named it *Æcidium Toddaliæ* Petch. He mistook the æciod uredia for æcia, and at the time of distributing the rust in Sydow's *Fungi exotici exicatti* he rectified the error and named it *Uredo Toddaliæ* Petch. In 1920 Sydow (1919) placed it in his newly established genus *Ctenoderma* of which the type was *Ctenoderma cristata* (Speg.) Syd. on *Cupania sp.* from south America. Four species of *Ctenoderma* had been established by 1939.

Mains (1939) who critically examined some new collections of the rust on *Cupania sp.* discovered that in addition to the two spore forms described as pycniospores and teliospores respectively, there is yet another spore form of the rust. Mains showed that the spores taken to be teliospores by Sydow (1919) are in reality urediospores and that the third spore form found by him represented the telial stage. This resembled the Raciborskian genus *Skierka* in all respects. Accordingly he merged *Ctenoderma* in the older genus *Skierka*.

In the type and authentic specimens, in three out of four species of *Ctenoderma*, Mains was able to see the telial stage and he transferred them to *Skierka*. With regard to the fourth species *Ctenoderma Toddaliæ*, he did not find any teliospores. The urediospores of this rust bore a good resemblance to those of the other species of the genus, but in the absence of the telial stage, he did not transfer the rust to *Skierka* but retained the name *Ctenoderma Toddaliæ*, thinking it best to leave the species until the discovery of the telia under that name. Now that the telial stage has been discovered by the writer in the present investigation it is clear that Mains did well in not transferring the rust to *Skierka* for the telial stage bears no resemblance to the telial stage of that genus.

The rust on *Toddalia aculeata* Pers. was first observed by the writer in 1940 at Balehonnur on *Toddalia aculeata* var. *floribunda*. Fluffy white

sori were observed on either surfaces of the leaves consisting of pycnia and uredia. The structure and the measurements of the urediospores indicated that it was the same rust collected by Petch (1908), under the name *Ædidium Toddaliæ*. On the *T. aculeata* var. *gracilis* growing round-about Bangalore stray cases of infection were also observed. Morphological studies of the rust was made in detail and the collections were made all the year round to see if the teliospores develop. In some of the collections made in the



FIGS. 1–4

Fig. 1. Leaf of *Toddalia aculeata* showing infection spots. × Nat. Size. Fig. 2. Young pycnium showing the concentration of hyphæ within the epidermis. × 400. Fig. 3. Mature pycnium showing ostiole. × 400. Fig. 4. Section through an uredium. × 400.

month of January and March telia were observed associated with uredia. The infected patches show slight hypertrophy, and on these pycnia and uredia are distributed on either surfaces. Following secondary infections of the urediospores the infection becomes very sparse. The secondary uredia do not show any hypertrophy. The telia which are sometimes associated with secondary uredia are in short *Cronartium*-like columns white and pulverulent. Telia have been noticed only on the *gracilis* variety.

Pycnia

Pycnia are amphigenous distributed in concentric patches mixed up with uredia. Macroscopically they appear as tiny yellow specks. They are applanate and intraepidermal (Fig. 3). The pycnial initials are formed by the concentration of hyphæ within the epidermis (Fig. 2). The cells become enlarged in size and as development proceeds they become distended, and get disorganised. The mature pycnium might extend up to three to four epidermal cells. A palisade layer of pycniosporophores is formed. The cuticle ruptures opening out a narrow ostiole. A large number of hyphæ line the inner surface of the ostiole, but no conspicuous ostiolar paraphyses are present, though few of them emerge out of the ostiole. Nectar is secreted out of the ostiole embedding the pycniospores. The pycnia on *Toddalia aculeata* var. *gracilis* measures 130-87 × 26–56 μ, and those on the *floribunda* variety 192–270 × 76–96 μ. Intraepidermal pycinia have been reported in *Mainsia* by Jackson (1931) where he states that they were neither subepidermal nor subcuticular but between the two.

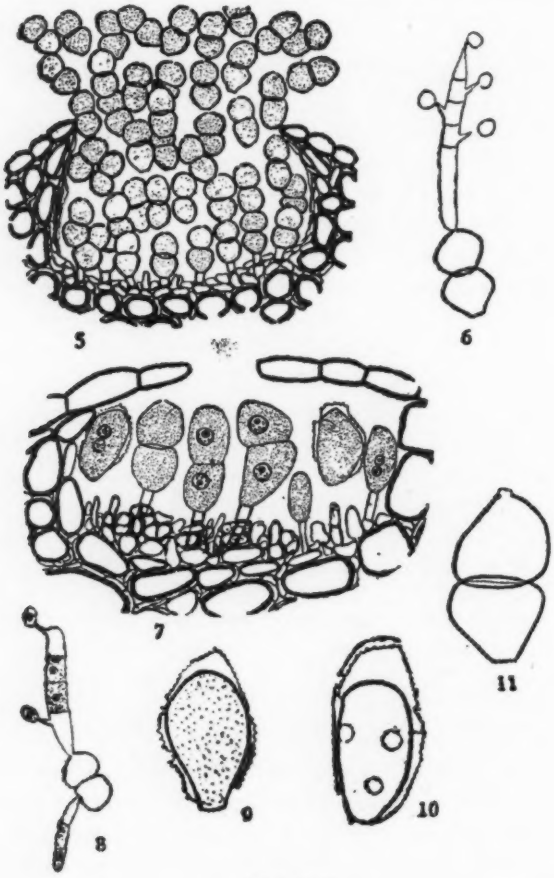
Uredia

Uredia are subepidermal (Fig. 7) erumpent, and covered over by the epidermis in early stages. The urediospores are stipitate, the stalk being hyaline thin-walled measuring up to 46 μ in length. The spores are oblong ellipsoid, cinnamom-yellow and angular due to unequal thickening of the exospore. Serrate lateral ridges are present on the sides and at the apex except in the region of the hilum (Figs. 9 and 10). The spores are binucleate with three to four indistinct germ pores. The urediospores of the *gracilis* variety measures 34–54 × 24–26 μ. and those of *floribunda* 44–60 × 19–27 μ.

The urediospores were germinated and stained by the method suggested by the writer (1940). It is very difficult to obtain successful germination, and the spores retain viability for quite a long time. The urediospores infect the same host and develop secondary uredia, which structurally resemble the primary ones. But the primary uredia are associated with pycnia developing on hypertrophied patches, whereas the secondary uredia are minute, without causing hypertrophy and associated with telia.

Telia

Telia are hypophyllous, minute and erumpent. They arise when they are fully developed as short *Cronartium*-like columns measuring up to 1·5 to 2 mm. in length (Fig. 6). They are white and fluffy in consistency. Telia are subepidermal the sorus developing deep within the host tissue. From the base of the sorus teliospores are abstricted in succession. These



FIGS. 5–11

Fig. 5. Section through a telium showing catenulate teliospores. × 240. Fig. 6. Germinating teliospore showing basidiospores. × 300. Fig. 7. Teliospores developing in old uredium, × 400. Fig. 8. Germinating teliospore. × 240. Fig. 9. Urediospore showing lateral ridges of the exospore. × 800. Fig. 10. Urediospore showing three germ pores. × 800. Fig. 11. Mature teliospore showing the sessile nature. × 800.

rupture the epidermis and on account of close adpression are exserted out in short columns. The basal spore is borne on a basal cell, which however is not present in the mature spore at the top.

The teliospores are two-celled, hyaline, and sessile. There is a single distinct germ pore in each cell (Fig. 11) and two germ pores have rarely been noticed in the upper cell. This is probably an abnormal feature. Some of the teliospores retain short basal cells. The spores are binucleate in early stages, and in mature spores the nuclei fuse forming a syncaryon.

Teliospores germinate within the sorus soon after maturity. Spores scraped from mature telium had germinating spores in spite of the fact that dry weather prevailed for many days before the collection of the material. However mature spores that had not germinated within the sori were collected and germinated on slides. They were found to germinate readily and develop sporidia within 6 hours (Figs. 6 and 8). The sporidia are ovate, and uni-nucleate, measuirng $28 \times 8\ \mu$. Teliospores have not been observed for the *floribunda* variety.

Conclusions

The systematic position of the rust on *Toddalia aculeata* which have been placed under various genera by different workers has to be reconsidered in the light of the present work. Sydow (1919) placed it in the genus *Ctenoderma* mistaking the urediospores for teliospores. And Mains who merged the genus *Ctenoderma* with *Skierka* following the discovery of telia for the rust on *Cupania* surmised that the rust on *Toddalia aculeata* might also be a species of *Skierka*, owing to the close resemblance of the urediospores in the two forms. The two-celled, sessile teliospores arising in *Cronartium*-like columns present in the rust on *Toddalia aculeata* indicates that it does not belong to the genus *Skierka*.

Puccinia Toddaliæ Raciborski has been recorded on *Toddalia aculeata* from Java by Raciborski (1909). The rust is a microcyclic form, with teliospores which are reported to be pedicellate, the pedicel measuring up to $50 \times 13\ \mu$ in length.

The rust on *Toddalia aculeata* under study differs from *Puccinia Toddaliæ* in having sessile teliospores, which are exserted out in short columns. It can be compared with only three genera, *viz.*, *Pucciniosira* Lagerh., *Coleopuccinia* Pat, and *Didymopsora* Diet. In *Pucciniosira* the pycnia are deeply sunk, the telia have a peridium, and intercalary cells are present between the teliopsore cells, and the latter have no germ pores. These characters are different from those on the rust on *Toddalia*.

Only telia are known in *Coleopuccinia*. The two-celled spores are abstricted off in chains and they are embedded in a gelatinous matrix which is a prominent feature. The two-celled *Puccinia*-like spores separate easily in one of the species giving the impression that they are one-celled. There are no germ pores. The absence of any gelatinous secretion in the *Toddalia* rust and other characters exclude it from this genus.

In the genus *Didymopsora* the teliospores are two-celled arising in short *Cronartium*-like columns, and are characterised by the absence of any pseudoperidia. The pycina are subepidermal distributed round the telia. The four species so far recorded are known to be microcyclic. They have been known to occur only in South America, and have not been recorded anywhere else, and of course not in India.

The absence of any peridia in the telium, and occurrence of columnar teliospores which germinate immediately in *Didymopsora* compares favourably with the rust on *Toddalia aculeata*. The pycnia are amphigenous and intraepidermal in the *Toddalia* rust, whereas they are reported to be subepidermal in all the species of *Didymopsora* so far recorded. Further in the three species of *Didymopsora*, *D. Solani-argenti*, *D. Solani*, and *D. Chuquiraguæ*, the two cells of the teliospores are very fragile and get separated at maturity, a character not present in the *Toddalia* rust, and this feature might not have any significance for separating a new genus. Likewise in *Coleopuccinia simplex* Diet. the two cells of the spores get separated, whereas they remain persistent in *Coleopuccinia sinensis* Pat. The uredial stage has so far not been reported for *Didymopsora*, and has been discovered for the first time on *Toddalia*.

Didymopsora Toddaliæ Thirumalachar and Mundkur sp. nov.—Infection spot in round yellow patches, slightly hypertrophied. Pycnia amphigenous, intraepidermal, yellow, mixed up or surrounded by uredia, few hyphæ emerging out of the ostiole, applanate, 130–187 μ broad, 26–56 μ high, uredia amphigenous, erumpent, subepidermal and aparaphysate; urediospores stipitate, oblong-ellipsoid, slightly angular, exospore ridged at the sides and at the apex, minutely verrucose, germ pores three and indistinct, measuring 34–54 $\times$ 24–26 μ. Telia hypophyllous, minute, white, in short columns, measuring about 1·5 to 2 mm. long, subepidermal, teliospores abstricted off in chains, spores closely adpressed and emerging out in short columns, no peridium; teliospores two-celled, sessile, hyaline or translucent, thin-walled, constricted at the septa, single distinct germ pore in each cell, spores germinating soon after maturity within the sorus, measuring 45–60 $\times$ 19–27 μ. Promycelium 4-celled, basidiospores thin-walled, germinating *in situ*, measuring 28 $\times$ 8 μ.

Habitat.—On leaves and rarely on petioles of *Toddalia aculeata* var. *gracilis*, leg. M. J. Thirumalachar, Yashvantapur, Bangalore, Mysore State, 19–3–1942.

Type deposited in Herb. Crypt. Ind. Orient of the Imperial Agricultural Research Institute, New Delhi, India.

Maculis contagium orbicularibus, flavis, leniter tumidulis. Pycnia amphigenia, intraepidermalia, flava, Urediis cincta vel intermixta, paucis hyphis per ostiola emergentibus, applanatus 130–180 μ latus, usque 25–56 μ altus. Uredia albida, amphigena, erumpentia, subepidermalia aparaphysata; urediospori pedicellati, oblongo-ellipsoidi, leniter angulares, exospori ad apicem et latus jugati, minutati verruculosi, 3–pori germinales indistincti, magnitudinis 34–54 $\times$ 24–26 μ. Telia hypophylla, minutissima albida, in brevisculis columellis, 1·5–2 mm. altis, subepidermicis; teliospori catenulis adstricti, sine peridio 2-cellulares, spori post maturitatem statim germinantes intra sorum, 45–60 $\times$ 19–27 μ. magnitudinis, promycelium typice quadri cellulare, basidiospori leptodermi, *in situ* germinantes, magnitudinis 28 $\times$ 8 μ.

Habitat.—In vivis foliis *Toddalia aculeata* Pers. var. *gracilis* leg. M. J. Thirumalachar, Yashvantapur, Bangalore, Mysore State, India 19–3–1942.

Didymopsora macrospora Mundkur and Thirumalachar sp. nov.—Infected patches slightly hypertrophied, yellow: pycnia amphigenous, arranged in concentric rings, applanate, intraepidermal, measuring 192–270 μ broad, 76–96 μ high. Uredia amphigenous, erumpent, white, subepidermal and aparaphysate; urediospores stipitate, oblong-ellipsoid, exospore unequally thickened to form lateral ridges, 44–60 $\times$ 17–24 μ, with three indistinct germ pores. Telia not known.

Habitat.—On leaves of *Toddalia aculeata* Pers. var. *floribunda*, leg. M. J. Thirumalachar, Balehonnur, Mysore State, India, 14–5–1940.

Maculis contagium leniter tumidulis, flavis; pycnia amphigena, annulis concentricis disposita, applanata, intraepidermalia, 192–270 μ lata, usque 76–96 μ alta; Uredia amphigenia, erumpentia, albida, subepidermica, et aparaphysata ; urediospori pedicellati, oblongo-ellipsoidi, angulares, exospori ad latus et apicem supercelio prædati, 44–60 $\times$ 17–24 μ magnitudinis, 3–pori germinationis indistincti. Telia adhuc ignota.

Habitat.—In vivis foliis *Toddalia aculeata* vqr. *floribunda*, leg. M. J. Thirumalachar, Balehonnur, Mysore State, India, 14–5–1940.

Type specimen deposited in the Herb. Crypt. Ind. Orient. of the Imperial Agricultural Research Institute, New Delhi, India.

Measurements of the pycnia and urediospores indicate that the rust on the *floribunda* variety of *Toddalia aculeata* has to be accommodated as a new species of *Didymopsora*. The pycnia are very much larger than those of the previous species, and the urediospores are also longer and narrower. The pycnia and uredia so closely resemble those of *Didymopsora Toddaliæ* in other respects that there is no doubt about the position of this rust in *Didymosora* even though the teliospores have not so far been discovered.

Summary

In the present paper an account of the morphology and life-history of two new species of *Didymopsora*, *D. Toddaliæ*, and *D. macrospora* is presented. The rust is parasitic on the leaves of *Toddalia aculeata* var. *gracilis* and *floribunda*.

The rust on *Toddalia aculeata* had previously been identified by Petch as *Aecidium Toddaliæ*, and later on changed to *Uredo Toddaliæ*. Sydow in 1920 transferred the rust to the genus *Ctenoderma* established by him as *C. Toddaliæ* (Petch) Syd. He mistook the urediospores for the teliospores. Mains who merged the genus *Ctenoderma* with *Skierka*, suggested that the rust on *Toddalia* might be a species of *Skierka*. In the present investigation, the telia have been observed, and the rust has been placed in the genus *Didymopsora*.

Pycnia are intraepidermal, applanate, without conspicuous ostiolar paraphyses. Uredia are subepidermal, and the urediospores are stipitate, with characteristic lateral ridges at the sides and at the apex.

Telia are exserted out in short *Cronartium*-like columns, and the spores are two-celled, sessile, hyaline, with a single distinct germ pore in each cell and germinating immediately. Telia have not been observed for *Didymopsora macrospora*.

In conclusion, the writer wishes to acknowledge his indebtedness to Dr. B. B. Mundkur, Imperial Agricultural Research Institute, for critically going through the manuscript, and valuable suggestions, and to Dr. L. N. Rao, Professor of Botany, Central College, Bangalore, for helpful suggestions and encouragement.

Postscript.—

The writer recorded two species of *Didymopsora*, *viz.*, *D. Toddaliæ* Thirumalachar and Mundkur, on *Toddalia aculeata* var. *gracilis*, and *D. macrospora* Mundkur and Thirumalachar on *T. aculeata* var. *floribunda*. At the time of establishing the new species, telia were however not observed for *D. macrospora*. The rust was placed in the genus *Didymopsora*

on account of the close similarity of the pycnia which are intraepidermal, and urediospores which possess lateral ridges formed by unequal thickening of the exospore.

The telia of *Didymopsora macrospora* have since been discovered in a collection of *Toddalia aculeata* var. *floribundu* made at Balehonnur on 13–9–1942. Both in structure and development they resemble those of *D. Toddaliæ*, and this fact has justified the placing of the rust in that genus even before their discovery.

The infection spots are yellow, slightly swollen, and measure up to 3 to 7 mm. in diam. Telia are subepidermal, deeply sunk in the host tissue, opening out by a narrow slit. Teliospores are exserted out in short columns. The exact length of the columns could not be measured because they were washed out during heavy rains. The basal spores have pedicels measuring up to 15–51 μ. Spores scraped from young telium retain their pedicels, and can be mistaken for pedicellate teliospores. But all the mature teliospores are sessile, hyaline or pale-yellow and very much constricted at the septa. In the scrapings of the teliospores the two cells get easily separated, indicating their fragile nature. As in *D. Toddaliæ*, the upper cell often posesses two germ pores. This was considered to be an abnormal feature, since they occurred in few cases. In *D. macrospora*, nearly 80% of the teliospores have two germ pores in the lower cell situated below the septa, whereas in all the teliospores of *D. Toddaliæ* only one germ pore was observed in the lower cell. The teliospores measure 51–65 $\times$ 18·8–31 μ, they germinate immediately and form basidiospores which are thin-walled, measuring about 24 $\times$ 12 μ.

It is manifest that pycnia, uredia and telia as also their respective spores of *D. macrospora* are larger than those of *D. Toddaliæ*, and this justifies the allocation of a separate species. Furthermore, the teliospores of *D. macrospora* possess long pedicels at the base of the hymenium, which appear however only as basal cells in *D. Toddaliæ*. The occurrence of two germ pores in the lower cells of the teliospore of *D. macrospora*, further distinguishes the two rusts. The descriptions of the pycina and uredia have already been given. A formal description of the telia is given here.

Telia hypophylla, minutissima, maculis contagium leniter tumidulis usque 3–7 mm. diam., in brevisculis columellis, subepidermicis, teliosporæ catenulis, adstricti, sine peridio, 2-cellularis, sessilis, hyalini, leptodermi, fundus sporis pedicellatis, usque 51 μ, in medio constricti, singuli disticti pori germinales in utrumque et 2 pors germ. in subter cellulis, sporis statim post maturitatim germinantes intrasorum, 45–60 $\times$ 19–27 μ magnitudinis, promycelium typice quadricellulare, basidiosporæ leptodermi, *in situ* germinantes, magnitudinis 28 $\times$ 8 μ.

Telia amphigenous, subepidermal, infection spot slightly hypertrophied, yellow, measuirng up to 3–7 mm. diam.; teliospores two-celled, in small columns, deeply constricted at the septa, hyaline, fragile, getting easily separated, sessile basal spores on pedicels, up to 51 μ, germ pores distinct, one in the upper cell, and two in the lower cell, germinating immediately within the sorus, measuring 51–65 $\times$ 18·8–31 μ; promycelium exserted out, typically 4-celled, basidiospores thin-walled, pyriform, measuring up to 24 $\times$ 12 μ.

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STUDIES IN INDIAN HEPATICÆ

III. On a New Species of *Nowellia, N. indica* Pandé et Srivastava from the Western Himalayas

By S. K. Pandé and T. N. Srivastava

(Botanical Laboratory, The University of Lucknow, Lucknow)

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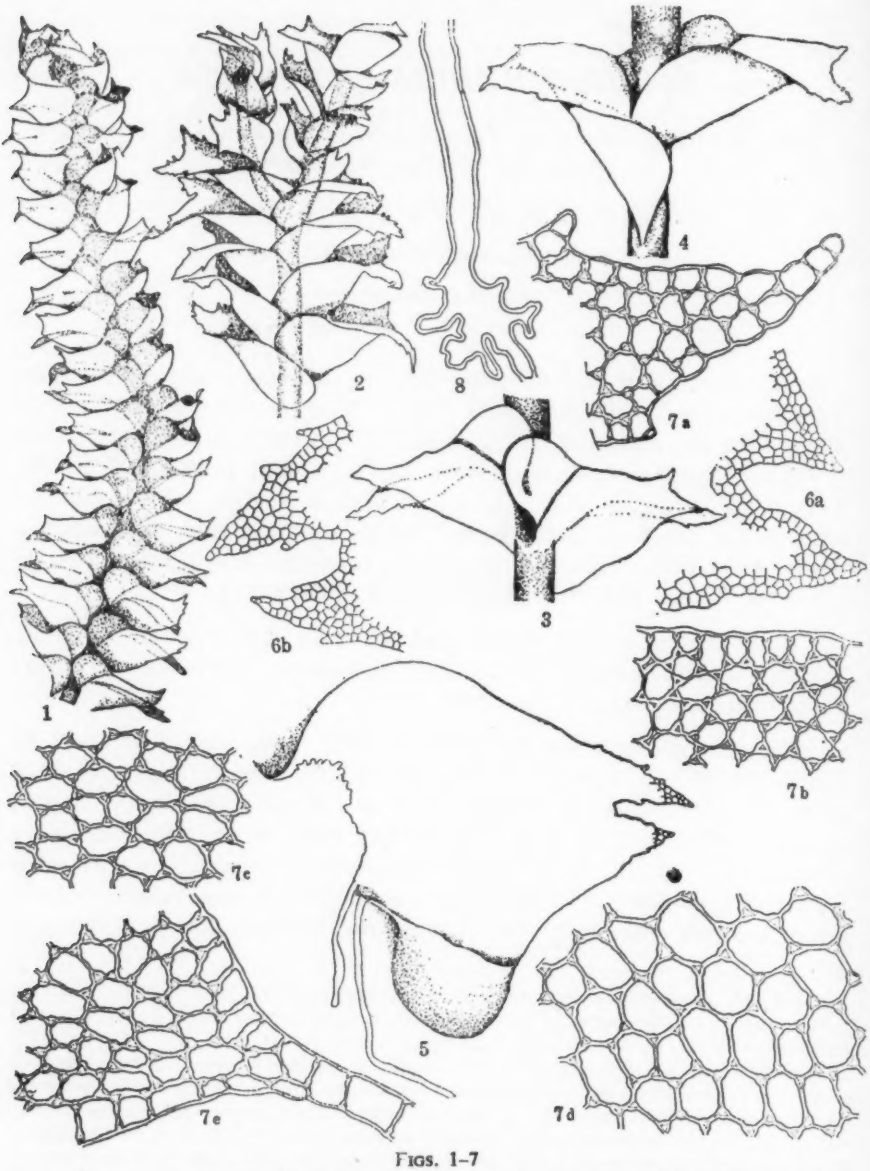
(Communicated by Prof. Birbal Sahni, F.R.S.)

The interesting liverwort *Nowellia* Mitt., as at present known, comprises 5 species (3, pp. 347–50; 4, p. 443). One of these, *N. caledonica* St., is endemic to New Caledonia, while *N. curvifolia* (Dicks.) Mitt. is widely distributed in Europe, North America and certain parts of Asia (2, p. 98 and 3, p. 348). Two of the remaining species, *N. dominicensis* St. and *N. Wrightii* (G.) St., occur in the islands of Dominica and Guadeloupe, the latter species is also known from Cuba. The fifth species *N. borneensis* St. is confined to the islands of Borneo and the Philippines (2, p. 98 and 3, p. 349).

In the present note, which was read before the 26th session of the Indian Science Congress (Lahore, 1939, p. 119), a new species of *Nowellia, N. indica** Pandé and Srivastava is described.

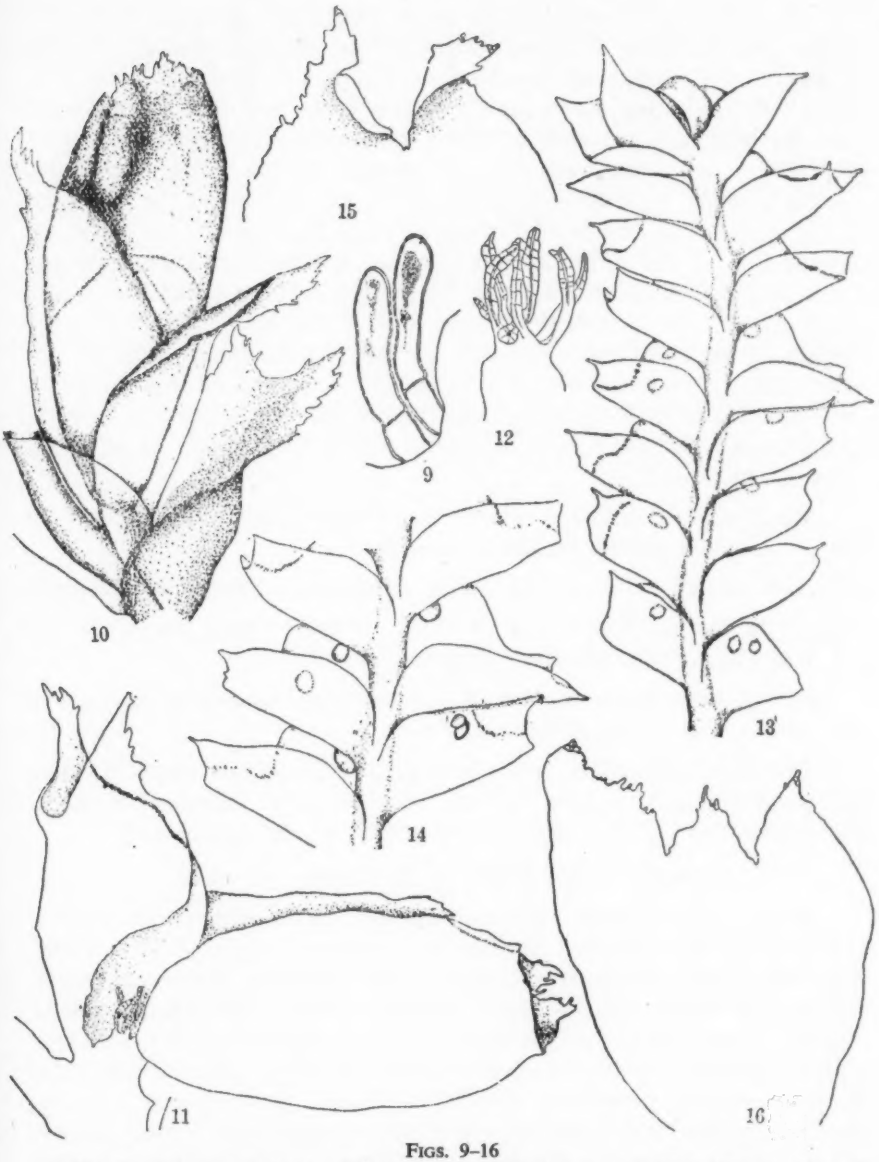
The material, on which this article is based, was collected by Dr. S. K. Pandé and Mr. R. N. Misra in October 1937 from near Girgaon (6,000′), about 67 miles north-east of Almora, in the Western Himalayas. It was identified and named by the authors in July 1938 and the MS. and figures were sent to Mr. W. E. Nicholson for favour of comparison with other species and opinion. Mr. Nicholson confirmed our observations and returned the MS. and figures but retained the specimen for his herbarium.

* Simultaneous with the publication of our observations (*vide Proc. Ind. Sci. Congress*, Lahore, 1939, p. 119) there appeared a note by Mr. R. S. Chopra (*vide* Chopra, R. S., "Notes on Indian Hepatics—II : Sikkim Himalayas and Bengal," *Proc. Ind. Acad. Sci.*, Vol. VIII, p. 43, Dec. 1938) wherein he describes a new species of *Nowellia, N. orientalis* Chopra. Unfortunately this plant is known only in the sterile condition and Mr. Chopra gives no illustrations to supplement his description. There is, however, a striking similarity in the vegetative characters of the two plants. May be that the two plants are identical ; but one can never be definite on the resemblance of the vegetative features alone, particularly since *N. indica*, as will be seen from the following description, shows some important deviations in certain characters from all the known species of the genus.



FIGS. 1–7

Fig. 1. A shoot, Ventral aspect, × 13. Fig. 2. Same as Fig. 1, Dorsal aspect, × 16·7. Fig. 3. Portion of stem for attachment of leaves, Ventral aspect, × 25. Fig. 4. As above, Dorsal aspect, × 25. Fig. 5. Leaf, Ventral view, × 40. Fig. 6 *a* and 6 *b*. Leaf apex, × 66·7. Fig. 7, Cells from leaf, × 250. (*a*. from apex, *b*. from margin, *c*. from middle, *d*. from basal region and *e*. from the lobule). Fig. 8. Rhizoid, × 250.



FIGS. 9–16

Fig. 9. Gemmæ, × 433·3. **Fig. 10.** Perianth, Ventral view, × 26·7. **Fig. 11.** Perianth dissected out to show archegonia, × 26·7. **Fig. 12.** Apex of the female shoot after removing the perianth and bracts to show archegonia, × 56·7. **Fig. 13.** Male shoot, × 40. **Fig. 14.** Portion of a male shoot, × 56·7. **Fig. 15.** Bract, × 26·7. **Fig. 16.** Perianth dissected out; the archegonia have not been drawn, × 26·7.

N. indica was found growing on a tree trunk in association with *Calypogeia alternifolia* Nees and *Plagiochila* sp. It can, however, be easily distinguished from both of these because of its darker colour and more robust habit. The individual plants grow to a length of about 2 cm. or more and may be irregularly branched. The leaves are inserted by a very narrow and almost transverse base with the postical margin being inflexed and saccate forming a lobule lying adpressed to the stem when the plants are dry (Fig. 1). The lobe is bifid (Figs. 2–5) and the segments are acute and dentate (Figs. 5 and 6) but not produced in two long capillary-hamate processes described to be characteristic of the genus (1, p. 278). The species is dioecious. The female inflorescence is cladogenous. The bracts are similar to the leaves but distichous and not tristichous as usual in the genus (the bracteole is absent). Antheridia are borne in short branches and there are 4–6 pairs of bracts (Figs. 13 and 14) with 1–4 antheridia in the axil of each. Globose oblong unicellular gemmæ (Fig. 9) are found at the apex of the shoot.

The liverwort described above shows following deviations from all the previously described species of *Nowellia*:—

(1) there are no bracteoles (amphigastria) in the female inflorescence,

(2) the leaves, though bifid to about 1/4 their length, are not drawn out into long capillary-hamate segments, and

(3) the male bracts are 1 to 4 androus, not monandrous as usual in the other species of the genus.

The fertile material of both the male and the female plants is rather scanty and the authors have included the plant under *Nowellia* because of the characteristic inflexed and saccate postical margin of the leaf.

Nowellia indica Pandé et Srivastava, sp. nov.

Dioica. plantæ foliosæ, robustæ, rigidulæ, viridis sæpe purpurascentes, corticolæ. *Caulis* validus, irregulariter ramosus. *Folia caulina* imbricata, brevi basi subtransverse inserta, valde concava, erecto-conniventia, postica late inflexa, saccata; apice emarginato-bifido, lobis acutis, dentatis. *Cellulæ*, superæ $14 \times 27\,\mu$, mediane $31 \times 24\,\mu$, basales $45 \times 30\,\mu$, parietibus validus, trigonis validus. *Cellulæ sacculæ* $22 \times 32\,\mu$. *Amphigastria* nulla. *Perianthia* magna, cylindrica. *Folia floralia* bijuga, conduplicatim, concava, profunde patula, foliis simillima, symmetrica æqualiterque bifida. *Andræcia* in ramulis terminalis, bracteis 1–4 androus ; *antheridia* ovalia, amphigastria nullas. *Gemmiparous*. Gemmæ ex apice vegetativæ, unicellulares. Reliqua desunt.

Habitat.—On bark. Girgaon (6,000 ft.), 67 miles N.E. of Almora, Western Himalayas, INDIA.

Collector.—S. K. Pandé and R. N. Misra.

Type-specimen.—Lucknow University Hepatic Herbarium No. 2029.

The authors beg to acknowledge their deep indebtedness to Prof. B. Sahni, D.SC., SC.D., F.R.S., for his keen interest and kind advice. Their grateful thanks are also due to Mr. W. E. Nicholson for some valuable suggestions.

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ON THE MULTIPERFORATE PLATES OCCURRING IN THE XYLEM VESSELS OF SOME MONOCOTYLEDONOUS ROOTS

By B. C. Kundu, M.A., Ph.D., F.L.S.
(Professor of Botany, Presidency College, Calcutta)

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Multiperforate plates have been observed in the xylem vessels of some dicotyledon stems and described by several botanists. They seem to form a common feature in the primitive vessel segments where the plates are usually more or less scalariform and are always found at the oblique end walls of vessels (Bailey and Tupper, Bliss, Frost, Jeffrey, Thompson, Tippo). They rarely form reticulate or similar other patterns and are not commonly found in the transverse end walls, but their occurrence in the transverse end walls of vessel segments has been reported by Solereder (1908), Smith (1938) and Majumdar (1939) in the stems of different species.

Von Mohl (cited by Tangl, 1871) has described net-like cross walls in the vessels of palm roots. Besides this, multiperforate plates in the vessels have not been, so far as the author is aware, reported in any other root, dicotyledon or monocotyledon. While preparing transverse sections for a class the author observed beautiful multiperforate plates in the metaxylem vessels of the roots of *Alocasia indica* Schott (Fig. 1, Pl. XIII). In macerated material also these plates are found to occur in abundance. Their occurrence has also been observed in some palm roots and certain other monocotyledon roots, *viz.*, *Colocasia antiquorum* Schott and certain other members of the Araceæ, *Crinum asiaticum* Linn., *Canna indica* Linn., etc. Further, vessels with multiperforate plates characterise the roots of *Michelia champaca* Linn., a primitive dicotyledon. They are also found occasionally in the vessels of the adventitious roots of *Tinospora cordifolia* Miers.

The nature of the perforations and their occurrence in the xylem vessels seem to be alike in the roots of *Alocasia*, *Colocasia* and other members of the Araceæ and *Crinum*. Further they show certain other structural peculiarities for which the multiperforate plates of the vessels of these plants will be mainly described in this paper, with occasional reference to *Canna indica* and others. Besides the perforation in their vessel segments palm roots

show certain other structural features for which it is desired to describe the anatomical characteristics of these roots in a subsequent paper.

It is very peculiar that none of the vessels of *Alocasia*, *Colocasia* and other members of the Araceæ and *Crinum* have simple perforations at their ends. All the vessels have got multiperforate end walls; in some cases the perforations are not quite prominent and the vessels look like so many tracheids; in *Canna indica*, however, a great number of vessels show scalariform or similar other plates at their ends, while only some have also simple perforations; in some cases a vessel segment may have simple perforation at one end and multiperforate plate at the other.

Usually three kinds of vessel segments can be found in these plants:—

(1) those having transverse or slightly inclined end walls at both ends (Fig. 1);

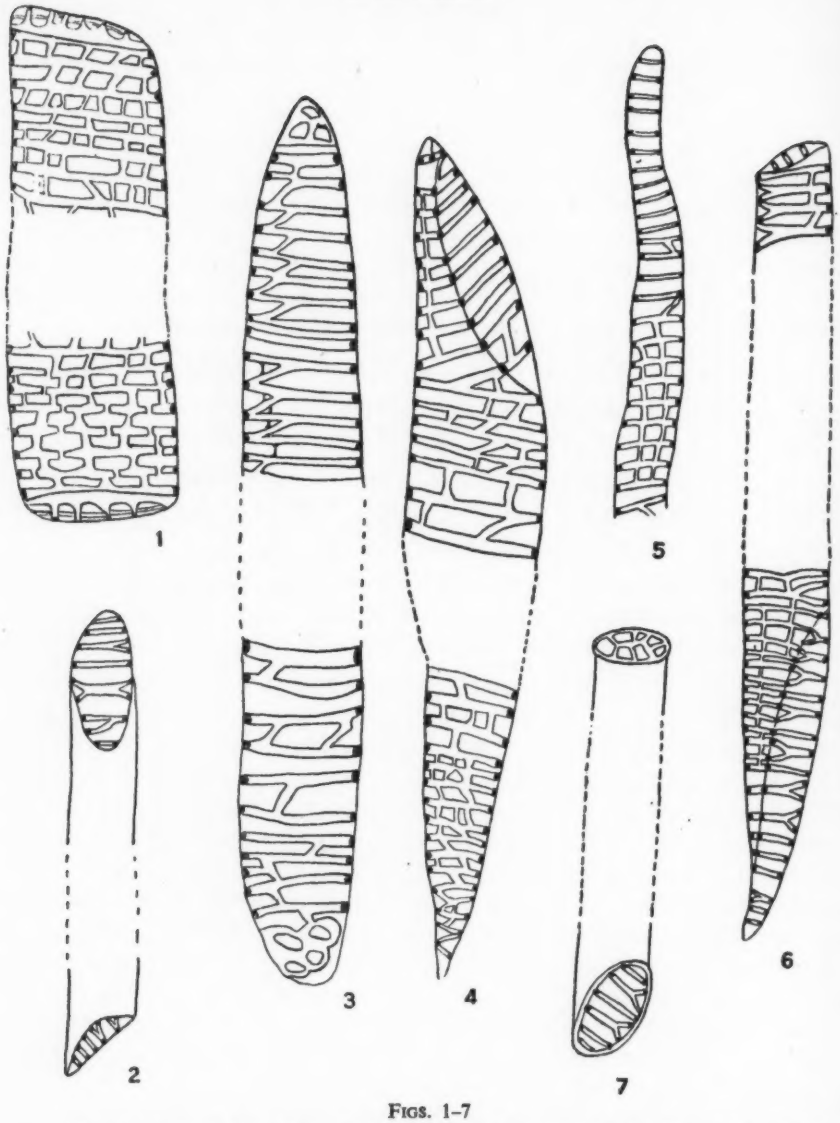
(2) those having oblique end walls at both ends (Figs. 2, 3, 4 and 5); and (3) those having transverse or slightly inclined end walls at one end and oblique end walls at the other end (Figs. 6, 7).

In the first case the vessel segments are placed one above the other; their ends are either concave or convex to the plane of perforation and the concave end of one fits nicely against the convex end of the other just above or below it. These types of vessels are particularly abundant in *Alocasia* and *Crinum*.

In the second case the segments are usually longer than those of the first type. The oblique end walls of adjacent vessel segments are closely fitted one against the other in such a fashion that the bars of the perforated plates of the two segments are placed one above the other, leaving the perforations quite free. In this category must be placed certain other longer and thinner segments with long pointed ends. They have the usual type of scalariform-reticulate thickening in their lateral walls but have no end walls and thus appear more or less tracheid-like. The long pointed ends of these segments are not often placed above others; they may end freely or may be arranged in a dovetailed fashion (Fig. 3, Pl. XIII). They are fairly abundant in all the species and may be regarded as the most primitive type of segments from which all other types have evolved.

The vessel segments of the third type may be regarded as a transitional form; these are also quite abundant in all the three species studied, but are not found in *Canna*.

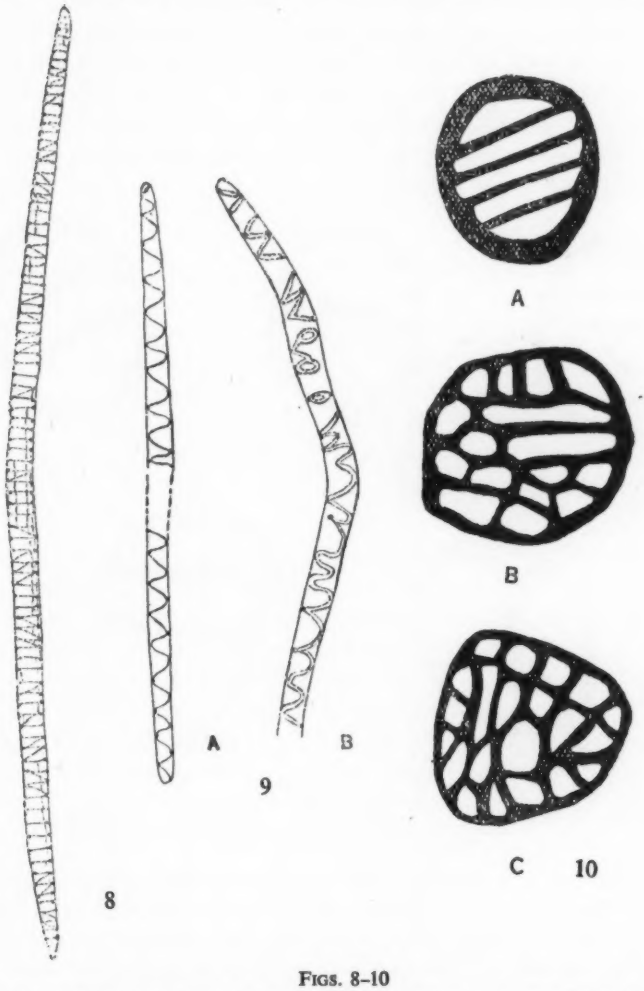
Spirally thickened elements predominate in the protoxylem and the segments are considerably elongated and have mostly pointed ends without



FIGS. 1–7

Fig. 1. A vessel from *Crinum asiaticum* showing transverse end walls. ×250. Figs. 2, 3 and 4. Three vessels from the root of *Crinum asiaticum* having oblique end walls. In Fig. 3 the end walls are not well differentiated and in Fig. 4 at one end of the vessel segment the end wall is not differentiated. Fig. 2. ×71. Figs. 3 and 4. ×250. Fig. 5. Peculiarly constructed vascular segment from the root of *Crinum asiaticum* with no end wall and having spiral thickening at the end ; only one end is shown. ×250. Figs. 6 and 7. Vessels from the roots of *Crinum asiaticum* having transverse or slightly inclined end wall at one end and oblique end wall at the other. Fig. 6. ×250. Fig. 7. ×71.

showing definite end-walls (Figs. 8, 9 A, 9 B). The metaxylem vessels all have the scalariform-reticulate type of thickening in their side walls.



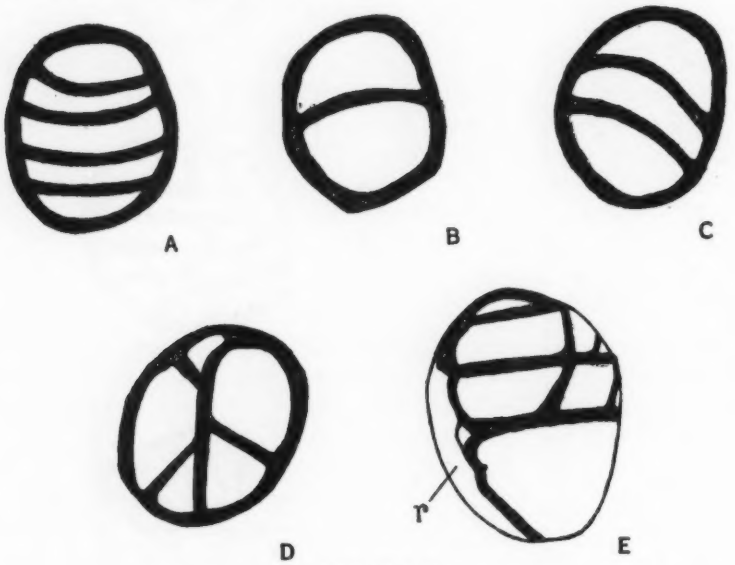
FIGS. 8–10

Fig. 8. A tracheid-like vascular element from *C. asiaticum* having pointed ends. × 72. Fig. 9 A and B. Two spirally thickened elements from *Alocasia indica* without end walls. Fig. 9 A. ×72. Fig. 9 B. × 280. Figs. 10 A, B, C. Multiperforate plates at the ends of the vessel segments from the roots of *C. asiaticum*. ×262.

The pattern of the multiperforate plates may be different in different vessel segments; it may be different at the two ends of the same segment (Fig. 7). It usually consists of transversely elongated bars which may run

parallel to one another at the end of the vessel segments (Fig. 10 A) but in the majority of cases these bars run more or less obliquely and often branch to form a more or less net-like perforation (Figs. 10 B and C; Fig. 1, Pl. XIII).

In *Canna indica* vessels usually have transverse or slightly inclined end walls and the bars at the ends of vessel segments in the majority of cases run more or less parallel to one another (Fig. 11 A). In this plant a single bar is often seen across the end walls of a vessel segment (Fig. 11 B); two bars are not quite uncommon (Fig. 11 C). Reticulate perforations are only occasionally found (Fig. 11 D, E).



FIGS. 11 A–E

Multiperforate plates in the vessel segments from the roots of *Canna indica*. *r*–rim of the perforation. ×262.

Occasionally in a transverse section a complicated net-like appearance can be seen (Figs. 5 and 6, Pl. XIII), and this is certainly due to the different orientation of two sets of bars and their branches at the end walls of two adjacent vessel segments and thus intersecting one another. This phenomenon has been observed and figured by Solereder (1908), Thompson (1923), Smith (1935) and Majumdar (1939) in the stems of different plants. Smith remarks that in *Helianthus annuus* this sort of appearance is a rare occurrence ; but in *Alocasia* and *Crinum* where a good number of vessels with transverse end walls have multiperforate plates one could expect these in thick sections.

From microchemical reactions the bars of these plates appear to be lignified. With aniline chloride and HCl and phloroglucin and HCl the bars and their branches give the same lignin reaction as do the scalariform-reticulate thickenings of the longitudinal walls of vessel segments. In maceration by 5% chromic acid or by Schultze's maceration method the bars of the plates as well as the scalariform-reticulate thickenings of the lateral walls of segments lose much of the lignin and do not give any strong lignin reaction; but they now give a cellulose reaction with iodine followed by sulphuric acid.

Discussion

Thompson (1923), Chalk (1933), Smith (1935) and others have regarded the occurrence of the multiperforate plates in the stems of plants examined by them as a rare phenomenon. Majumdar (1939) in view of their plentiful occurrence both in leaves and stems of *Heracleum Sphondyllium* does not regard them as abnormal types. These plates in the vessel segments of the roots of *Alocasia*, *Colocasia*, *Crinum*, *Canna* and other genera are quite common features. In fact, in *Alocasia*, *Colocasia* and *Crinum* they form the normal features as in these cases vessels with simple perforations have not been observed.

Certain features in relation to the evolution of vessels in angiosperms can be observed in the vessels of *Alocasia*, *Colocasia* and *Crinum*. Bailey and Tupper (1918) conclude that in the primitive type of vessels the segments will be long in view of the fact that there has always been great differences in length of tracheids and vessel segments in the different groups of plants considered to be primitive and derivative. Besides, primitive vessel segments will have oblique end walls and the more specialised ones transverse end walls. Here in *Alocasia*, *Colocasia* and *Crinum* we notice that the vessels with oblique end walls at both ends are usually longer than the other types and so these may be considered as primitive.

Botanists have tried to correlate the nature of the multiperforate plates with the pitting on the lateral walls of the same vessel segment. It has been admitted by most workers [Bailey and Tupper (1918), Bliss (1921), Frost (1930, 1932), Jeffrey (1917), Thompson (1918, 1923) and Tippo (1938)] that vessels with scalariform end walls are the most primitive, and angiospermic vessels with the single large perforation have been derived from those with scalariform perforation. Thompson (1918, 1923) and Frost (1930, 1932) regard that the primitive vessels with scalariform perforation plates are derived from scalariform tracheids of the fern type, and are thus characterised by scalariform lateral pitting. Frost (1930) further concludes that " there is

a high correlation between the primitive scalariform condition of end walls and the scalariform condition of side walls ".

On the other hand Boodle and Worsdell (1894), Jeffrey (1917), Bliss (1921) and MacDuffie (1921) conclude that the scalariform plates at the end walls of vessel segments of dicotyledons have originated by the fusion of horizontal rows of circular pits.

Although there has thus been difference of opinion regarding the origin of the multiperforate plates, most investigators are unanimous regarding the relation of the scalariform plates at the end walls with the type of pitting in the lateral walls of the same vessel segments.

The vessel segments of the roots studied are characterised by scalariform-reticulate pitting (except the protoxylem elements) in the side walls, but the plates at the end walls may be scalariform or scalariform with branched bars or reticulate. In segments with narrow ends the end walls are highly inclined or absent and as such do not materially change the tracheid-like shape of the primitive vessel segment. In all these cases it may be assumed that secondary thickening matters have been deposited throughout the inner walls of the vessel segments without any break at the end; so the same type of pitting is found in the lateral and in the end walls.

In discussing the origin of net-like perforations at the ends of vessel segments of some dicotyledon stems Bliss (1921) correlates them with the circular pits of lateral walls and says that they have originated by haphazard fusion of circular pits. She also considers that the scalariform perforation with branched bars is a special form of reticulate type of perforation. She has been supported by MacDuffie (1921). Brown (1918), Thompson (1923) and Frost (1930) regard that the reticulate type of perforation is " evidently an anomalous oddity resulting from the breaking down of vestigal scalariform perforations in scalariform porous woods ". In *Crinum*, *Colocasia* and *Alocasia* the reticulate types of perforations of the vessel segments can be correlated to the type of pitting in the lateral walls of some segments. The origin of these plates cannot be attributed to the fusion of circular pits as such pits are absent in the lateral walls. It is very likely that in some cases, at least, the reticulately perforated plates are formed by the breaking down of certain of the scalariform bars as has been suggested by Brown, Thompson and Frost.

Eames and MacDaniels (1925) do not give any definite views regarding the origin and nature of these plates. Majumdar (1939) who has recently observed similar types of multiperforate plates both in spiral (protoxylem) vessels and pitted (metaxylem) vessels of stems and leaves of *Heracleum*

Sphondyllium remarks that it would seem a waste of time to correlate the type of multiperforate plates with the characteristic pitting of the lateral walls of vessel segments. He thinks that these are found as " occasional sporadic depositions determined by the tendency of the vessel protoplast to form cellulose lamellæ at its surface ".

Conclusion

On account of the nature of the perforation plates in the vessel segments of these roots the writer cannot regard them as occasional sporadic depositions. He definitely believes that there is a correlation between the bars of the plates and the pitting of the lateral walls of vessel segments and the condition is quite in conformity with views expressed by Frost. It is admitted by all workers that the roots of plants are characterised by primitive features. Here are some roots which show not only their usual primitive feature but are further characterised by a very primitive type of vessels.

From a study of the second type of vessel segments somewhat of the nature of tracheids, the writer further thinks that the metaxylem vessels of roots have developed phylogenetically from a typical scalariform tracheid by the loss of pit membranes at the end of the tracheid and in this respect he supports the views expressed by Frost (1930) in connection with the development of vessels in the stems of arboreal dicotyledons. He cannot, however, support the conclusions of Bliss (1921) that the primitive type of vessels in angiosperms is pitted and has been derived from the pitted tracheids.

In the roots of *Alocasia*, *Colocasia* and *Crinum* we find very primitive vessel segments having the nature of tracheids and also rather advanced types of segments having transverse end walls, although in every case simple perforation is absent. In *Canna indica* the vessels seem to be more specialised as most of the segments have transverse end walls and in a number of them the perforations are simple.

Summary

The occurrence and structure of multiperforate plates in the xylem vessels of the roots of some monocotyledons are described. Such plates are present in all the vessel segments of the roots of many members of the family Araceæ and in *Crinum asiaticum*. In all these plants vessel segments with simple perforations have not been observed. The vessel segments as found in these roots may be of three types: (i) those having oblique end walls at both ends; (ii) those having oblique end wall at one end and

transverse or slightly inclined end wall at the other; (iii) those having transverse or slightly inclined end wall at both ends. The perforation plates of these segments may be scalariform, scalariform with branched bars, or reticulate. The bars of the plates can be correlated to the scalariform-reticulate thickening of the lateral walls of the vessel segments. These types of vessel segments are regarded as forming a primitive feature. Further it is concluded that the xylem vessels of roots have developed phylogenetically from typical scalariform tracheids by the loss of pit membranes at their ends.

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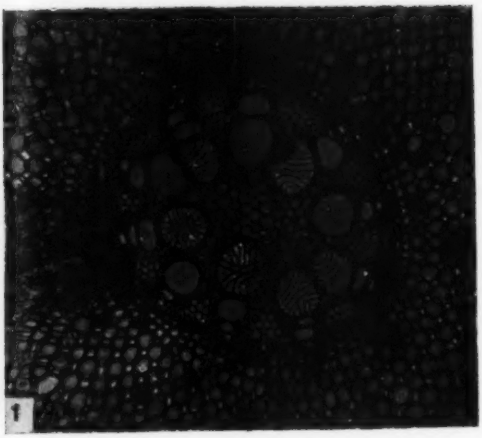
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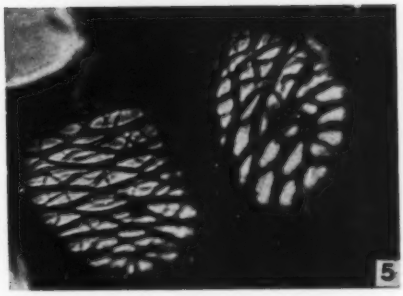
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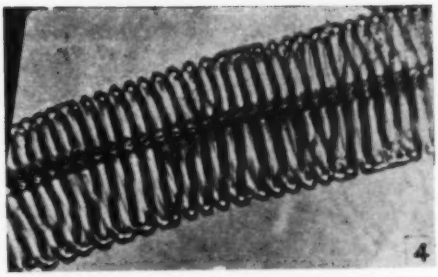
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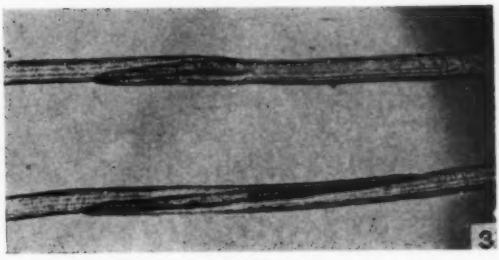
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EXPLANATION OF PLATE FIGURES

FIG. 1.—Transverse section of a root of *Alocasia indica* showing 6 multiperforate plates in the vessel segments of the metaxylem. ×46.

FIG. 2.—A number of vessel segments from the root of *Crinum asiaticum* showing the two ends, ×15.

FIG. 3.—Figure showing the arrangement of adjacent vessel segments with long oblique ends falling one upon the other (from macerated material). ×46.

FIG. 4.—The arrangement shown in Fig. 3 in high power. ×182.

FIGS. 5 and 6.—Examples of complicated net-like appearances due to the superposition of two sets of bars (in *Alocasia indica*). ×260.

THE MECHANISM OF β-AMYLASE INHIBITION BY VITAMIN C

BY P. SESHAGIRIRAO AND K. V. GIRI

(From the Biochemical Laboratory, Andhra University, Waltair)

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THE fundamental rôle which vitamin C plays in normal metabolism has not yet been fully elucidated. The fact that vitamin C is widely distributed in plants and animal tissues has naturally attracted attention to the question of a possible biochemical relationship between the vitamin and enzymes. As the physiological properties of the vitamin appear to depend essentially upon its high reducing property and the reversible nature of its oxidation, it has been supposed that it plays an important rôle as catalyst in biological oxidations. But attempts to establish a relationship between the vitamin and dehydrogenase systems have not been successful. Although there have been many such investigations on the influence of vitamin C on other enzymes, but little work has been reported on the mechanism of the reaction.

In a series of papers published by Giri (1937; 1938 *a*, *b*; 1939) on the interaction of vitamin C and phosphatases of both plant and animal origin, evidence was presented in favour of the view that the inhibition of the activity of the enzymes by vitamin C is related to the oxidation of the vitamin. There was considerable experimental support for this view and the findings were subsequently confirmed in other laboratories (Courtois, 1939). It occurred to us that it might be possible to obtain further evidence in favour of the view by studying the behaviour of other enzymes towards the vitamin. The present investigation was, therefore, undertaken with a view to examining the mechanism of the action of vitamin C on amylases.

Purr (1934) in his work on the influence of vitamin C on plant and animal amylases, showed that the vitamin has an inhibiting effect on the β-type of plant amylase and has no effect on the α-type. He further showed that the β-type of animal amylase is activated by the vitamin. Later, several workers have reported the inhibiting action of vitamin C on β-amylase of barley (Hanes, 1935; Janicki, 1939), wheat [Euler *et al.* (1934)] and sweet potato (Giri, 1941).

As regards the mechanisim of inhibition, Hanes (1935) has put forward the view that the inhibiting effect of vitamin C is due to the presence of the dienol grouping in the vitamin and that it is not due merely to the reducing

property of this grouping since it was observed that two other dienol compounds, dihydroxymaleic acid and reductone also exert the same type of inhibition. It was further shown that the inhibition by a dienol compound can be reversed by oxidative destruction of the dienol grouping and also by the addition of a variety of other reducing substances like hydrogen cyanide, sodium hydrosulphite and sulphydryl compounds. Janicki (1939), on the other hand, reports that the inhibition is associated with a dehydrogenation of the ascorbic acid, a view which was first postulated by Giri (*loc. cit.*) in his studies on the interaction of vitamin C and phosphatases. It is clear, therefore, that further critical study is required of the effect of vitamin C on the amylases, and of the mechanism of the reaction. The observations recorded in the present paper on the influence of vitamin C on β-amylase from sweet potato lend further experimental support to the view that inactivation of enzymes by vitamin C is related to the oxidation of the vitamin.

Experimental

Preparation of the enzyme.—The amylase was prepared from sweet potatoes (*Ipomoea batata*) by the method described by Giri (1934 *a*). Fresh tubers were washed well with water, minced and dried in the sun. The dried material was then powdered and stored in bottles. The enzyme was obtained in solution by extraction of one part of dry powder in five parts of water saturated with toluene for twenty-four hours in the refrigerator. After filtration the extract was centrifuged and dialysed in collodion bags for twenty hours against distilled water, which was frequently renewed at short intervals. The precipitate which formed was centirfuged off. In this manner clear solutions were obtained, which retained the activity for long periods when stored in the refrigerator. This enzyme has been shown to be a pure β-amylase (Giri, 1934 *b*; 1938 *c*).

Merck's "soluble starch" was used as substrate for the determination of the activity of the enzyme.

The B.D.H. sample of ascorbic acid was used throughout this work. The vitamin was estimated by titration with iodine, after the addition of glacial acetic acid.

All the substances employed in the present investigation were of the highest grade of purity, either Merck's or Kahlbaum's products.

Water distilled from an all pyrex still was used in the preparation of all reagents, substrate and vitamin C solution.

The activity of the amylase was determined by assay of the reducing power of maltose formed in aliquots withdrawn from the reaction

mixture at different time intervals according to the method of Willstätter and Schüdel (1918). The digestion mixtures contained 20 c.c. acetate buffer M/5 (P_H 5·6), 20 c.c. 2 per cent. starch solution and other substances, whose influence on the activity is to be determined, the total volume being adjusted to 50 c.c. unless otherwise stated. For the determination of the activity of the amylase treated with vitamin C, oxalic acid (10 mg.) was added to the digestion mixture in order to stabilise the vitamin from oxidation. The significance of the addition of a stabiliser such as oxalic acid to the digestion mixture is discussed in the text of the paper. Suitable blanks were always run for each experiment. The incubation temperature was 30 ± 0°·1 C. The activity is always expressed in mg. maltose formed in the total volume of the digestion mixture.

The P_H of all the solutions used in testing their influence on the activity of the enzyme, was always adjusted to the P_H of the experimental solution (P_H 5·6). In the case of those substances (particularly purine derivatives) which are difficultly soluble in water, they were dissolved first in minimum amount of alkali and diluted to the required strength. These solutions, when added in small amounts to the reaction mixture, were found to have no significant influence on the P_H of the solution.

All the solutions of the substances and of ascorbic acid were always freshly prepared for each experiment.

The inhibition of the enzymic hydrolysis of starch by vitamin C.— Previous investigations (Giri, *loc. cit.*) suggested the possibility that the inhibiting action of vitamin C on the enzymic hydrolysis of starch might be connected with its oxidation by Cu^{++} catalysis. It was therefore of interest to investigate the effect of addition of vitamin C alone and with Cu^{++}, on the rate of hydrolysis of starch and also the influence of stabilisers which protect the vitamin against oxidation.

The following mixtures were set up containing:—

1.	40 c.c. M/5 acetate buffer P_H 5·6	40 c.c. starch 2 per cent.	
2.	do.	do.	10 mg. vitamin C
3.	do.	do.	10 mg. vitamin C; $CuSO_4$ 5 H_2O solution containing 10·7 γ Cu^{++}
4.	do.	do.	10 mg. vitamin C $CuSO_4$ 5 H_2O containing 10·7 γ Cu^{++} Oxalic acid (20 mg.)

The total volume of the reaction mixture in each case was made upto 98 c.c. with water. 2 c.c. of the enzyme solution was then added and the progress of maltose production was determined. The vitamin was always

added into the reaction mixture just before the enzyme was added. At stated intervals, 10 c.c. aliquots were removed for the determination of maltose and vitamin C respectively. The results are presented in graph form in Figs. 1 *a* and 1 *b*.

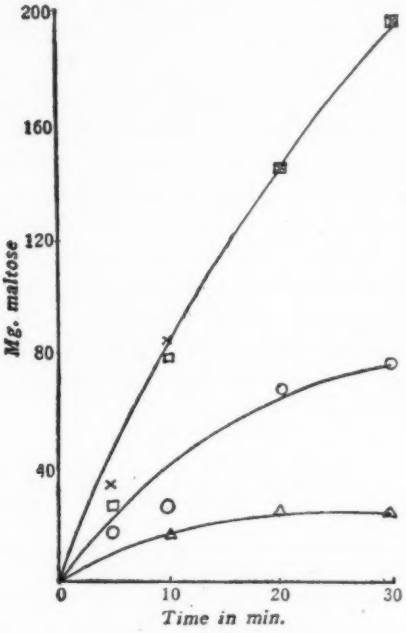

FIG. 1 *a*
Hydrolysis of Starch by the Amylase

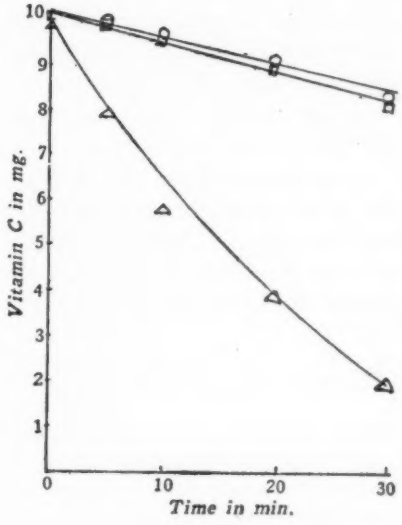

FIG. 1 *b*
Oxidation of Vitamin C

FIG. 1 *a, b*. The Influence of Vitamin C and Vitamin C-Cu$^{\cdot\cdot}$ Complex on the Enzymic Hydrolysis of Starch

×——× Enzyme + Substrate
○——○ Enzyme + Substrate + Vitamin C
△——△ Enzyme + Substrate + Vitamin C-Cu$^{\cdot\cdot}$
□——□ Enzyme + Substrate + Vitamin C-Cu$^{\cdot\cdot}$ + Oxalic Acid

Examining the curves of the hydrolysis course, it is clear that vitamin C–Cu complex inhibits the hydrolysis to a marked extent. Oxalic acid, which is found to protect the vitamin against oxidation (Krishnamurthy and Giri, 1941), prevents the inhibition by the complex. The curves relating to the oxidation of the vitamin indicate a rapid destruction of the vitamin in the digestion mixture containing vitamin C–Cu complex, while very little oxidation of the vitamin was noticed in oxalic acid mixture containing vitamin C and Cu^{++}. From these results it can be concluded that the complete absence of inhibition observed, when oxalic acid was added

to the digestion mixture, is due to the stabilising action of oxalic acid against the catalytic oxidation of the vitamin, and that the inhibition by vitamin C–Cu complex is related to the oxidation of the vitamin. It was also observed that under exactly similar experimental conditions Cu^{++} alone has no significant influence on the hydrolysis.

In the case of the inhibition observed by vitamin C alone although the rate of oxidation of the vitamin is of the same order as that of the vitamin in presence of Cu^{++} and oxalic acid, the hydrolysis is inhibited by the vitamin. This apparent discrepancy will be referred to later in this paper.

The influence of various stabilisers of vitamin C oxidation on the inhibition of enzymic hydrolysis of starch by vitamin C–Cu complex.— It has already been noted that a number of purine derivatives yeast nucleic acid and iminazole compounds inhibit the catalytic oxidation of vitamin C by Cu^{++} (Giri and Seshagirirao, 1942). The effect of these compounds, together with those which are known to protect vitamin C against oxidation, on the inhibition of the enzymic hydrolysis of starch by vitamin C–Cu complex was next investigated. Several typical experiments showing the extent of the prevention of the inhibition are summarised in Table I.

TABLE I

Influence of stabilisers on vitamin C–Cu inhibition of the hydrolysis of starch by β-amylase

The digestion mixtures contained 20 c.c. of acetate buffer; 20 c.c. starch (2 per cent.); vitamin C (5 mg.); $CuSO_4$ 5 H_2O containing 10·7γ Cu. $^{++}$. The stabilisers were added to the digestion mixtures in concentrations stated in the table. The total volume of the mixtures amounted to 50 c.c.

	Stabiliser added	Concentration of the stabiliser added	Amylase activity: Time of hydrolysis in minutes	
			15	30
Enzyme+substrate	..	..	95–108	151–159
Enzyme+substrate+vitamin C–Cu	..	..	13	22
do.	Xanthine	$26·3\times10^{-4}$ M	99	146
do.	Uric acid	$23·8\times10^{-4}$ M	95	146
do.	Guanine	$26·5\times10^{-4}$ M	103	151
do.	Theophylline	$22·2\times10^{-4}$ M	89	119
do.	Creatinine	$17·7\times10^{-4}$ M	47	77
do.	Histidine	$25·8\times10^{-4}$ M	103	151
do.	Cystine	$16·3\times10^{-4}$ M	86	129
do.	Oxalic acid	$31·7\times10^{-4}$ M	99	155

The results indicate that the inhibition of the hydrolysis by vitamin C–Cu complex is completely or partly annulled by the compounds tested. In the concentrations used in the above experiments the compounds were found

to have no appreciable influence on the hydrolysis of starch by the amylase, and on the determination of maltose production in the digestion mixture. It was also found that these compounds completely annul the inhibition caused by vitamin C alone. The foregoing experiments are sufficient to establish the fact that the inhibition of the enzymic hydrolysis of starch by the vitamin C–Cu complex is related to its oxidation, and those compounds which prevent the oxidation of the vitamin, also annul the inhibition of the hydrolysis by the vitamin C–Cu complex.

The influence of vitamin C concentration on the degree of inhibition.—In Table II are presented the results of experiments on the inhibition of the hydrolysis of starch by the amylase in presence of varying concentrations of the vitamin.

TABLE II

Influence of varying concentrations of vitamin C on the hydrolysis of strach by the amylase

The digestion mixtures contained 20 c.c. buffer ; 20 c.c. starch (2 per cent.) ; 1 c.c. enzyme and varying amounts of vitamin C. The total volume in each case was made up to 50 c.c.

Concentration of the vitamin mg.	Activity in mg. maltose formed in the total volume of the digestion mixture after 30 min. hydrolysis
0	98
0·1	77
0·2	64
0·5	56
1·0	43
5·0	30

The results show that there is gradual increase in the degree of inhibition with increase in the concentration of the vitamin in the digestion mixture.

The Influence of the oxidation products of vitamin C on the hydrolysis of starch by the amylase.—The oxidation products of vitamin C were prepared by oxidising the vitamin with Cu^{++} in presence of acetate buffer. A mixture containing 60 c.c. acetate buffer M/5 (P_H 5·6), 9 c.c. $CuSO_4$ 5 H_2O solution containing 32·1 γ Cu^{++}, and 12 c.c. vitamin C solution (15 mg.) was prepared and kept at room temperature (31° C.) for about 2½ hours until the vitamin was completely oxidised, as indicated by the absence of any reducing power. The effect of addition of this mixture on the enzymic hydrolysis of starch was tested and compared with that of the influence of an equivalent amount of vitamin C (5 mg.) and $CuSO_4$ 5 H_2O (10·7 γ Cu^{++}) on the hydrolysis.

The following digestion mixtures were set up :—

1.	20 c.c. starch (2 per cent.)	20 c.c. acetate buffer	9 c.c. water
2.	do.	do.	3 c.c. $CuSO_4$ 5 H_2O (10·7 γ Cu ++) 5 c.c. vitamin C (5 mg.) 1 c.c. water
3.	do.	27 c.c. of the mixture containing the oxidation products of vitamin C	2 c.c. water
4.	do.	do.	2 c.c. oxalic acid (10 mg.)

The digestion mixtures 3 and 4 contain the oxidation products formed from 5 mg. of vitamin C. 1 c.c. of the enzyme was added to each of the digestion mixtures and the maltose production was determined. The results are presented in Table III.

TABLE III

Inhibition produced by vitamin C–Cu complex, the oxidation products of the vitamin and the influence of oxalic acid

	Activity after 30 min. hydrolysis
1. Amylase + starch	88
2. Amylase + starch + vitamin C–Cu	6
3. Amylase + starch + oxidation products of vitamin C	31
4. Amylase + starch + oxidised products of vitamin C + oxalic acid..	88

The results show that the products formed by the catalytic oxidation of vitamin C by Cu^{++} have an inhibiting action upon the hydrolysis. Thus in addition to the inhibition of the hydrolysis caused by vitamin C alone the enzymic hydrolysis undergoes a second inhibition by the oxidation products of the vitamin. This second inhibition can also be annulled by oxalic acid and other compounds (*ex.*, xanthine, guanine, uric acid, nucleic acid, etc.). The greater inhibition observed in presence of vitamin C–Cu complex is probably the result of these two types of inhibition, namely, the primary inhibition caused by the vitamin and the secondary inhibition caused by the oxidation products of the vitamin. Both these two types of inhibition are annulled by oxalic acid and other stabilisers of vitamin C oxidation.

The influence of vitamin C on the amylase in the absence of the substrate.—The results of the foregoing experiments have shown that vitamin C alone and vitamin C–Cu inhibit the hydrolysis of starch by the amylase, the inhibition being greater in the latter case. For studying the effect of the vitamin on the enzyme in the absence of substrate, it is therefore necessary to prevent further inhibition by the vitamin in the digestion mixture. This can be achieved by adding a stabiliser to the digestion mixture to inhibit the oxidation of the vitamin. That this procedure is essential is

clear from the figures presented in Table IV which relates to the experiments on the influence of vitamin C alone on the enzyme in the absence of the substrate, when the activity is determined in presence and absence of the stabiliser (oxalic acid) in the digestion mixture.

TABLE IV

The influence of the addition of a stabiliser to the digestion mixture on the activity of the amylase treated with vitamin C

The experimental mixture contained 10 c.c. enzyme ; 4 c.c. buffer P_H 5·6 ; 3 c.c. vitamin C (5 mg.) and 3 c.c. water. As soon as the vitamin was added 2 c.c. of the mixture were removed for the estimation of the activity, which was determined in presence and absence of oxalic acid in the digestion mixture. This activity corresponds to 0 min. time. After 30 min. incubation at 30° C. another aliquot was removed and the activity determined.

Time of incubation of the enzyme with vitamin C	Activity	
	Without stabiliser	With stabiliser
0	54	109
30	51	101
Untreated enzyme	..	107

It is clear from the results that the inhibition observed is mainly due to the inhibition caused in the digestion mixture by the vitamin, and not due to the incubation of the enzyme with vitamin C. Under the experimental conditions described, vitamin C alone has very little influence on the enzyme. This is attributed to the presence of protective constituents in the enzyme solution which stabilise the vitamin against oxidation. This will be discussed later. The fact that the activity observed immediately after the addition of the vitamin to the enzyme is low indicates that the inhibition is mainly due to the influence of the vitamin in the digestion mixture, and this can be completely eliminated by adding a stabiliser. In subsequent experiments on the influence of vitamin C on the enzyme, the use of oxalic acid as a stabiliser to prevent or retard the oxidation of vitamin C in the digestion, mixture, is an expedient which is employed in the present investigation in order to annul the inhibition due to the oxidation of the vitamin. This was not accomplished in the work of other investigators and the absence of such stabilisers in the digestion mixture introduces a serious, and apparently insuperable, difficulty in the subsequent attempts to study the influence of the vitamin on the enzyme in the absence of substrate.

The relation between the oxidation of vitamin C and the inactivation of the enzyme treated with vitamin in presence and absence of Cu^{++} *and stabiliser.*—The following mixtures were set up to contain:—

1.	30 c.c. enzyme +	12 c.c. acetate buffer P_H 5·6 +	18·0 c.c. water
2.	do.	do.	9·0 c.c. vitamin C (15 mg.) 9·0 c.c. water
3.	do.	do.	9·0 c.c. vitamin C 0·9 c.c. $CuSO_4$ 5 H_2O solution containing 32·1 γ Cu++ 8·1 c.c. water
4.	do.	do.	9·0 c.c. vitamin C (15 mg.) 0·9 c.c. $CuSO_4$ 5 H_2O solution containing 32·1 γ Cu ++ 6·0 c.c. oxalic acid (30 mg.) 2·1 c.c. water

The mixtures were incubated at 30° C., and at stated intervals of time 2 c.c. and 5 c.c. of the mixture were removed for the determination of the activity and vitamin C content respectively. The experimental data are plotted in Figs. 2 *a* and 2 *b*.

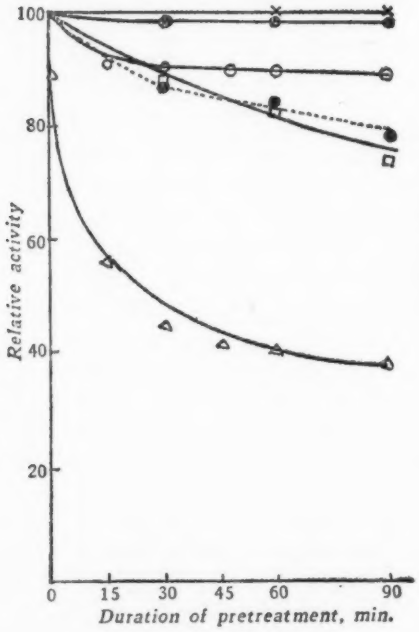

FIG. 2 *a*

Inactivation of the Amylase

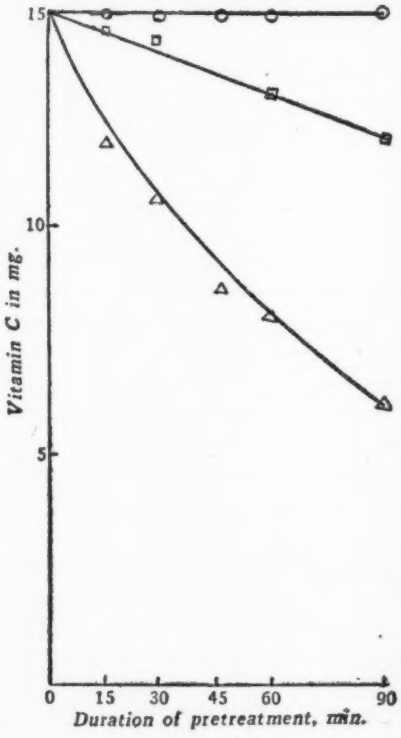

FIG. 2 *b*

Oxidation of Vitamin C

FIG. 2 *a*, *b*. The time course of inactivation of the Amylase by Vitamin C, Vitamin C-Cu, the oxidation products of Vitamin C, and the effect of Stabiliser

×——× Enzyme only

○——○ Enzyme + Vitamin C

△——△ Enzyme + Vitamin C + Cu··

□——□ Enzyme + Vitamin C + Cu·· + Oxalic Acid

●- - -● Enzyme + Oxidised products of Vitamin C

●——● Enzyme + Oxidised products of Vitamin C + Oxalic Acid

It is clear that vitamin C alone has very little influence on the enzyme, the inactivation being less than 10 per cent. On the other hand, vitamin C–Cu complex exerts greater inactivation of the enzyme. In presence of oxalic acid, however, the enzyme is protected from inactivation by vitamin C–Cu complex. There is a well-marked correlation between the oxidation of vitamin C and the degree of inactivation of the enzyme by vitamin C–Cu complex. The feeble inactivation of the amylase by vitamin C alone compared to the inactivation exerted by vitamin C–Cu complex is due to the stability of the vitamin in presence of the enzyme solution, which protects the vitamin against oxidation as indicated in Table V.

TABLE V

The oxidation of vitamin C in presence and absence of the enzyme preparation

Experimental solution	1.	7 c.c. acetate buffer P_H 5·6	2 c.c. vitamin C	11 c.c. water
	2.	do.	do.	5 c.c. enzyme 6 c.c. water
	3.	do.	do.	10 c.c. enzyme 1 c.c. water

Temperature, 30° C.

Time in minutes	mg. vitamin C in the experimental solution		
	1 No enzyme added	2 5 c.c. of enzyme	3 10 c.c. of enzyme
0	5·0	5·0	5·0
15	4·0	4·7	4·8
30	3·3	4·6	4·7
60	1·1	4·3	4·7

The influence of the oxidation products of vitamin C on the amylase in the absence of substrate.—The oxidation products of vitamin C were prepared as before by oxidising the vitamin in presence of Cu^{++}. The following experimental mixtures were set up.—

1.	20 c.c. enzyme solution	+	12 c.c. buffer P_H 5·6	+	18 c.c. water
2.	do.	+	24 c.c. of the oxidised products from 15 mg. vitamin C	+	6 c.c. water
3.	do.	+	do.	+	6 c.c. oxalic acid (30 mg)

The experimental solutions were incubated at 32° C. and at known intervals of time 2 c.c. of the solution were removed for the determination of the activity. The results are presented in graph form in Fig. 2 *a*.

It is clear from the curves that the oxidised products of vitamin C inactivates the enzyme, and that oxalic acid exerts complete protection

against the inactivation. Further examination of the curves in Fig. 2 *a* reveals the fact that the sum tqtal inactivation caused by vitamin C alone and the oxidised products of vitamin C is less than 50 per cent. of the inactivation induced by vitamin C–Cu complex. This difference is probably due to the oxidative inactivation of the enzyme induced by the catalytic oxidation of the vitamin by Cu.

The influence of various stabilisers of vitamin C oxidation on the inactivation of the amylase by vitamin C–Cu complex.—Attention has already been drawn to the fact that all those compounds which inhibit the oxidation of vitamin C by Cu, also annul the inhibition of the hydrolysis of starch by the amylase. Whether the same type of protection against the inactivation of the enzyme in the absence of substrate by vitamin C-Cu complex, is exerted by the compounds, was examined and the results of these experiments are presented in Table VI.

TABLE VI

The influence of stabilisers on the inactivation of the amylase by vitamin C-Cu complex

The experimental mixtures contained 10 c.c. of the enzyme; 4 c.c. of acetate buffer (P_H 5·6); 10·7 γ Cu^{++} as $CuSO_4$ 5 H_2O; 5 mg. vitamin C with and without the stabiliser. The total volume was always made up to 20 c.c. At stated intervals of time 2 c.c. were removed for the determination of the activity. Temperature of incubation, 30° C.

	Stabiliser added	Concentration of stabiliser 10^{-4} M	Incubation time in min.	Activity in mg. maltose formed after 30 min. hydrolysis	Percentage inactivation
Enzyme untreated	Nil			107	
Enzyme + vitamin C–Cu ..	Nil		15 30 60	68·6 53·6 42·9	36 50 60
do. ..	Oxalic acid	19·8	15 30 60	103 92 81·5	4 14 24
do. ..	Uric acid	14·9	15 30 60	107 103 103	0 4 4
do. ..	Xanthine	16·4	15 30 60	107 105 105	0 2 2
do. ..	Guanine	16·5	15 30 60	107 105 105	0 2 2

TABLE VI—(*Contd.*)

	Stabiliser added	Concentration of stabiliser 10^{-4} M	Incubation time in min.	Activity in mg. maltose formed after 30 min. hydrolysis	Percentage inactivation
Enzyme + vitamin C–Cu ..	Theophylline	13·9	15 30 60	107 103 103	0 4 4
do. ..	Yeast nucleic acid	0·025%	15 30 60	107 101 101	0 6 6
do. ..	Creatinine	22·1	15 30 60	107 97 92	0 10 14
do. ..	Cysteine	20·6	15 30 60	107 103 101	0 4 6
do. ..	Cystine	10·4	15 30 60	98 98 79	8 8 26
do. ..	Histidine	16·1	15 30 60	98 86 75	8 20 30
do. ..	Glutathione	8·1	15 30 60	107 107 105	0 0 2
do. ..	Potassium cyanide	15·3	15 30 60	107 105 105	0 2 2
do. ..	Hydrogen sulphide	1 c.c. saturated solution	15 30 60	107 105 105	0 2 2

The results clearly indicate that all those compounds, which are known to protect the vitamin against the catalytic oxidation by Cu^{++}, also protect the enzyme against inactivation by the complex. We have also observed that the feeble inactivation of the enzyme by vitamin C alone, is also annulled by these compounds.

Discussion

The results reported here indicate that vitamin C alone and vitamin C–Cu complex (i) inhibit the hydrolysis of starch by the amylase (Figs. 1 *a* and 1 *b*) and (ii) inactivate the enzyme in the absence of the substrate

(Figs. 2 *a* and 2 *b*). The inhibition and inactivation are completely prevented or abolished by a variety of compounds (Tables I and VI), which are found to protect the vitamin against catalytic oxidation by Cu^{++}. These compounds are oxalic acid, uric acid, xanthine, guanine, theophylline, yeast nucleic acid, creatinine, cysteine, cystine, histidine, glutathione, potassium cyanide and hydrogen sulphide (Giri and Seshagirirao, 1942). Although vitamin C alone has inhibiting action on the hydrolysis of starch, it has very slight effect on the enzyme in the absence of substrate. Vitamin C–Cu complex, on the other hand, not only inhibits the enzymic hydrolysis of starch but also inactivates the enzyme to a very marked extent. The inhibition of the hydrolysis of starch by vitamin C alone appears to be connected with its oxidation by traces of Cu^{++} present in the reaction mixture. The possibility that the inhibition may be due to the adsorption or complex formation of the vitamin with the substrate, starch colloid, the complex thus formed being less easily attacked than the free starch, cannot be eliminated. The present data, however, are not ample to emphasise exactly which of the above two factors are responsible for the inhibition observed in the digestion mixture by vitamin C. The very feeble inactivation of the enzyme by vitamin C alone (Figs. 2 *a* and 2 *b*) may be attributed to the presence of certain constituents in the enzyme solution which exert protection against the oxidation of the vitamin (Table V) thereby preventing the further inactivation caused by its oxidation.

In addition to vitamin C and vitamin C–Cu complex, the oxidation products of the vitamin obtained by oxidation with Cu^{++} also exert inhibition of the hydrolysis of starch by the amylase and inactivate the enzyme. The compounds which protect the vitamin against oxidation are also found to annul these two types of inhibition and inactivation (Table III and Fig. 2 *a*). Further work is in progress to test which of the substances present in the oxidation products of the vitamin is responsible for the observed inhibition.

These facts clearly demonstrate that both the inhibition of the hydrolysis of starch and the inactivation of the enzyme by vitamin C are to a great extent essentially related to the oxidation of the vitamin. The chief similarity between the substances serving as protectors against the inhibition and the inactivation by the vitamin is that they are all of the group of substances which inhibit the oxidation of vitamin C by Cu^{++}. This is certainly to be considered as a strong argument in favour of the view that the inhibition and the inactivation are induced by the oxidation of the vitamin. It is probably a case of induced oxidative inactivation of the enzyme by vitamin C–Cu complex. This view, however, may not be applicable to the feeble inactivation of the enzyme observed by vitamin C alone,

when the vitamin is not oxidised (Fig. 2 *a*). This feeble inactivation may probably be attributed to the influence of the dienol grouping on the enzyme as suggested by Hanes (1935).

It is worthy to emphasise here the importance of abolishing the development of inhibition in the digestion mixture, when studying the effect of the vitamin on the enzyme in the absence of substrate. This has been achieved by adding a stabiliser to the digestion mixture which serves to inhibit the oxidation of the vitamin, thereby completely preventing the development of further inhibition in the digestion mixture. This procedure is very essential in order to restrict the observations on the influence of the vitamin on the enzyme, without overlapping the inhibition in the digestion mixture.

In a paper published by Hanes (1935) sulphydryl compounds have been described as reversing the inhibition by vitamin C. In the light of the results obtained in the present investigation, it is probable that the observed effects have been due simply to the inhibiting action of the vitamin in the digestion mixture, which is eliminated by the substances added along with the enzyme. Thus it is probably a case of protection exerted by the compound against the inhibition of the hydrolysis by vitamin C rather than a reversal of inhibition. Further work is in progress to decide finally which of the two mechanisms stated above is correct.

The results obtained in the present study have an interesting physiological significance. If the activity of the amylase is to proceed in the plant cell, there must be some mechanism for protecting the oxidation of vitamin C by Cu^{++}. In all living cells purines occur either free or combined in the form of nucleic acids. They are widely distributed in nature. Vitamin C and Cu^{++} also occur together in many plant tissues. Since vitamin C–Cu complex and the oxidation products of the vitamin are shown to inactivate the amylase, they would be detrimental to the proper functioning of the enzyme and the function of the purine derivatives, nucleic acid, oxalic acid and thiol compounds is to protect the enzyme from such destruction. These results, therefore, are of interest in that they reveal another important physiological function of these compounds, namely they protect the vitamin against oxidation thereby regulating the activity of the enzyme in the living cell.

Summary

A critical study of the mechanism of β-amylase inhibition by vitamin C has been made. The hydrolysis of starch by the amylase is inhibited by vitamin C alone and vitamin C–Cu complex, the latter exerting greater inhibition.

The oxidised products of the vitamin prepared by Cu oxidation, also exert inhibition of the hydrolysis of starch by the amylase.

In addition to the inhibition exerted on the hydrolysis, vitamin C–Cu complex and the oxidised products of the vitamin inactivate the enzyme in the absence of substrate. Vitamin C alone brings about very feeble inactivation of the enzyme.

A variety of compounds—oxalic acid, uric acid, xanthine, guanine, theophylline, yeast nucleic acid, creatinine, cysteine, cystine, glutathione, histidine, potassium cyanide and hydrogen sulphide, which are shown to inhibit the catalytic oxidation of vitamin C by Cu^{++} annul the inhibition and the inactivation caused by vitamin C, vitamin C–Cu complex and the oxidation products of the vitamin.

A suggestive correlation between the inhibition of the hydrolysis and the inactivation of the enzyme on the one hand, and the oxidation of vitamin C on the other, has been established.

The physiological significance of these findings in their relation to the influence exerted by the purine derivatives and other stabilisers of vitamin C oxidation in regulating the activity of amylases in plant life is discussed.

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THE DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND FLOSS IN *BOMBAX MALABARICUM* D.C.

By I. Banerji

(*Department of Botany, Calcutta University*)

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Bombax malabaricum commonly known as the "silk cotton tree", is a native of the tropics. The tree sheds its leaves towards the end of the year and the bright red flowers appear next February or March, followed by the appearance of new leaves. The plant is grown in India chiefly for its floss which is used for stuffing cushions. According to Kirtikar and Bose[7] different parts of the plant are of medicinal importance.

The genus *Bombax* has been placed under the family *Bombacaceæ* by Hutchinson[5] and also by Engler and Prantl.[3] This family has not received the same attention from embryologists as the nearly related ones. Recently Thirumalachar and Khan[8] have given an account of the megasporogenesis and endosperm formation in *Eriodendron anfractuosum*. Our knowledge of the embryology of the other members of the family is as yet incomplete.

Material for this investigation was obtained from a tree growing in the University College compound. Ovaries in various stages of development were carefully dissected out from the flower buds and fixed either in Navaschin's or Allen's modified Bouin's fluid. For the study of the development of the floss ovaries from opened flowers and young bolls were fixed. It was necessary to trim away the superfluous tissue before fixation. The customary practice of dehydration, clearing, etc., was followed and sections were cut 8 to 16 microns thick. Sections were stained in Heidenhain's iron-alum hæmatoxylin.

Observation

(*a*) *The development of the female gametophyte.*—The ovary of *Bombax malabaricum* is pentalocular. The ovules arise as minute papillate processes from the axile placenta and very soon curvature is initiated. By the time the macrospores are in diakinesis the ovules become typically anatropous in form. They are bitegmic, the tips of the integuments are somewhat

massive. Both the integuments take part in the formation of the micropyle which is not straight but somewhat zig-zag (Text-Fig. 10).

The archesporial cell is hypodermal in origin. More than one archesporial cell have been noted in some preparations. Generally one of them develops. Two megaspore mother cells lying either side by side, or one above the other have been noted in some preparations (Text-Figs. 2 and 3). The 'cover cells' undergo repeated divisions as a result of which the megaspore mother cell is pushed considerably inside the nucellus (Text-Fig. 1). The megaspore mother cell increases in size before division. After the completion of division I, the protoplast passes through an interkinetic stage and then dyads are produced. The II division results in the formation of a linear tetrad of megaspores (Text-Fig. 4). The chalazal megaspore functions and the rest degenerate. The functional megaspore by three sucessive divisions produces an eight-nucleate embryo-sac, which is very much elongated and shows distinct polarity. The nucellar cells surrounding the embryo-sac show signs of degenration from the four-nucleate stage onwards. Thus the mature embryo-sac comes to lie a few layers below the nucellar cells and is closely surrounded by the integuments.

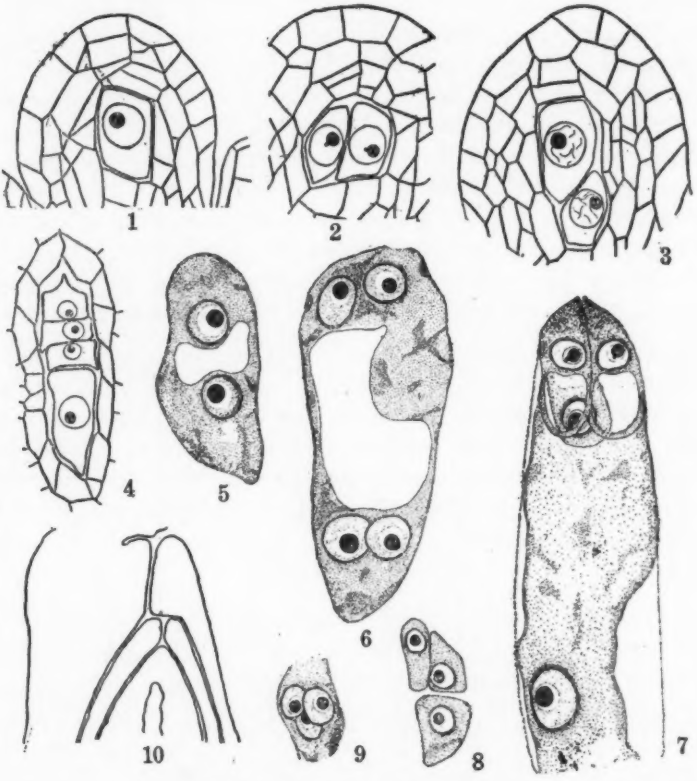
The mature embryo-sac is of the normal angiospermous type. The synergids are somewhat pear-shaped and have distinct vacuoles at their bases. The egg is elongated, it lies below the synergids and shows the usual structure. The secondary nucleus lies at the centre of the embryo-sac (Text-Fig. 7). The antipodals have cytoplasmic membranes; they are small in size, ephemeral and three in number (Text-Fig. 8).

The pollen tube enters the embryo-sac through the micropyle. The synergids degenerate after the entry of the pollen tube. The egg becomes very much elongated. The development of the embryo appears to be initiated long after endosperm formation. The primary endosperm nucleus divides very soon after fertilisation and a large number of free nuclei are produced which become distributed along the pheriphery of the sac. Wall formation takes place at a late stage. The endosperm nuclei show the presence of two or more nucleoli.

The process of fertilisation has not been observed, but a triple fusion nucleus was observed in one preparation, from this it might be inferred that in some ovules the fusion of the polar nuclei is not complete before fertilisation (Text-Fig. 9).

(*b*) *The development of the floss.*—It is interesting to note at the outset that the floss develops entirely from the epidermal layer of the inner carpellary wall of the fruit and the cells lining the radial walls take no part in

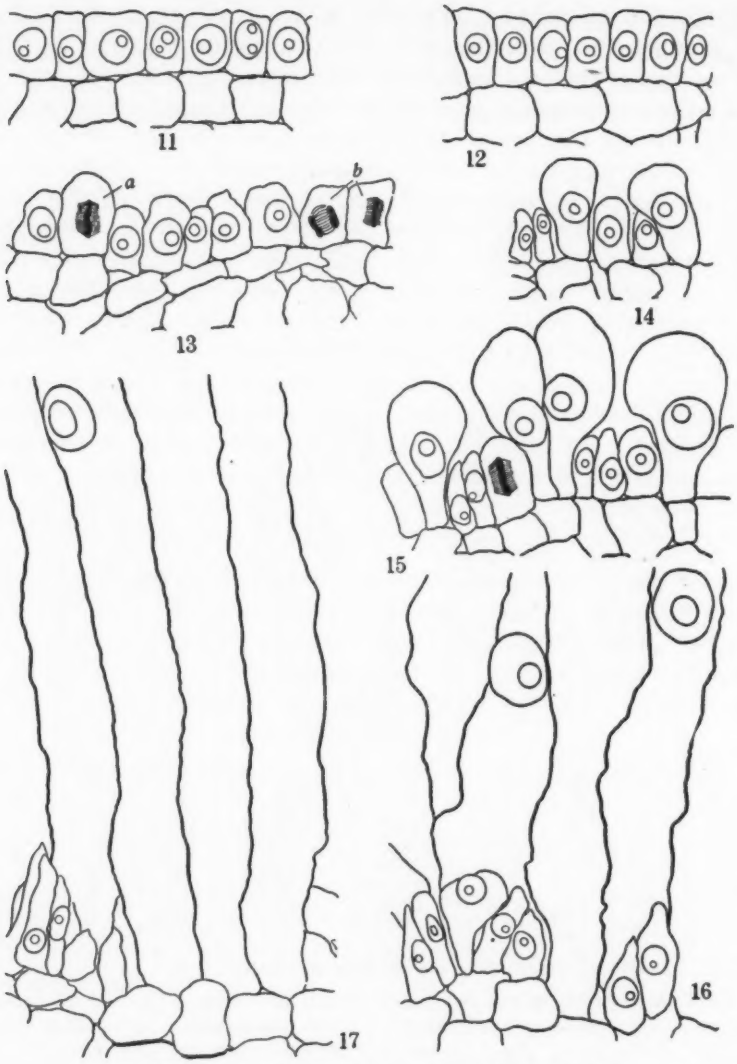
the process. Fibre development is generally initiated within a week after anthesis; at this time the endosprem cells form a lining layer around the embryo-sac, but the egg remains undivided. The cells destined to give rise to the fibres, lose their rectangular shape, become somewhat elongated and



Text-Figs. 1—10. *Bombax malabaricum* D.C.

Fig. 1. A megaspore mother cell pushed inwards due to the division of the cover cells. Figs. 2 and 3. Two megaspore mother cells lying side by side and one above the other. Fig. 4. A linear tetrad of megaspores. Fig. 5. Binucleate stage of the embryo-sac. Fig. 6. Quadrinucleate embryo-sac. Fig. 7. The mature embryo-sac showimg the egg apparatus and the secondary nucleus. Fig. 8. The three antipodal cells. Fig. 9. Triple fusion. Fig. 10. Section of the top of the ovule showing the zig-zag micropyle. Figs. 1 to 9. × 775. Fig. 10. × 75.

show the presence of dense cytoplasm and a single nuclues (Text-Fig. 12). Differentiation of these cells appears to be simultaneous throughout the epidermal layer of the inner carpellary wall, a few cells, however, remain



Text-Figs. 11—17. × 775. *Bombax malabaricum* D.C.

Fig. 11. The outer cells of the inner carpellary wall before initiation. Fig. 12. The form of the cells at the initial stages of fibre development. Fig. 13. Anticlinal division of the nuclei of the protruding fibre cells (*a*) and the ' non-fibre cells ' (*b*). Fig. 14. Fibre cells and undeveloped cells lying side by side. Fig. 15. A later stage in the development of the fibre. Note the undeveloped cells as also the anticlinal division in one of them. Fig. 16. Well developed fibre cells with groups of undeveloped cells at the base. Fig. 17 Illustrates the unconstricted nature of the base of the fibre cells.

inactive and interspersed in between groups of developing fibre cells (Text-Figs. 14 and 15). The initiation of fibre development is seen in the protrusion of the tips of the cells and the gradual increase in size of their nuclei. Vacuoles are commonly noted in the cells at this stage. Some of the elongating cells are also seen to divide anticlinally and result in the production of daughter cells (Text-Fig. 13 *a*). It appears that such cells soon become arrested in development. Division of the ' non-fibre cells ' is also noted at this stage, and as a result the fibre cells are pushed apart (Text-Fig. 13 *b*). These cells could be made out by the comparatively small size of their nuclei. Further development of the fibre cells takes place and along with it the cells become longer and their nuclei larger (Text-Fig. 15). Conspicuous vacuoles are noted above the nucleus at this stage. During the later stages of development of the fibres it becomes difficult to trace their outline as they overlap one another at their free ends. The nuclei of these cells are large and lie a little above the base of the cell (Text-Fig. 16). The mature fibres are uninucleate and unconstricted at the base (Text-Fig. 17).

The basal region of the mature fibres appears to be multicellular due to the anticlinal division and non-development of some of the epidermal cells of the inner carpellary wall (Text-Fig. 16). Evidence in this direction is obtained on examintion of fibres situated close to the radial walls of the carpel. In these fibres it is possible to trace their point of insertion and also their length for some distance. Text-Fig. 17 illustrates such a condition.

Discussion

The development of the female gametophyte in *Bombax malabaricum* shows no unusual features and appears to agree closely with the observations of Thirumalachar and Khan[8] on *Eriodendron anfractuosum*. Difference is noted, however, in the arrangement of the macrospores, but this cannot be considered to be of any significance, as in recent years both " T-shaped " and linear arrangement of macrospores have been observed in many plants. It is interesting to note that the micropyle shows similar orginization in both the genera. This might be a charesteristic feature of the family *Bombacaceæ*.

The development of the cotton fibre (*Gossypium* sp.) has been studied in detail by Gulati[4], Barritt[2] and others. It is only very recently that such investigation has been made in the family *Bombacaceæ* by Thirumalachar and Khan.[8] Their observations on *Eriodendron*, though agreeing in general with that observed in *Bombax*, differ in a few fundamental points. According to them, the cells of the floss are binucleate, the division of the nucleus

takes place during the early stages of the development of the fibre. Division of the nucleus of the elongating fibre cells of *Bombax* has been observed in many preparations, but the plane of division is always anticlinal and results in the production of daughter cells and not binucleate cells.

The presence of undeveloped cells at the basal region of the fibres has not been observed by Thirumalachar and Khan.[8] This appears to be a characteristic feauture of the present material. A reference to Fig. 12 in the paper of the above-mentioned authors, however, shows that some of the cells lying in between the incipient fibre cells are undeveloped. The next figure represents a group of uniformly developed binucleate fibre cells. It thus appears that all the cells of the inner epidermal layer of the carpel develop into floss in *Eriodendron anfractuosum.*

Another important observation made in the course of the present study is that fibre initiation and development take place simultaneously in all the five loculi of the ovary, with slight local differences. In this respect it differs from *Gossypium* sp. in whcih according to Ayyar and Ayyangar[1] the differentiation of the hairs proceeds for a number of days after flowering.

Jacob[6] has recently made an interesting observation in *Gossypium* sp. According to him the outer cells of the ovule which give rise to the fibres are binucleate in the early stages. After the fusion of the nuclei the fibre cells develop, *i.e.*, it is only the tetraploid cells that give rise to the fibres. Careful examination of the cells of the epidermal layer of the inner carpellary wall of the ovule and the boll during various stages of growth failed to reveal such a condition in *Bombax malabaricum.*

Summary

The paper gives an account of the development of the female gametophyte and floss in *Bombax malabaricum.*

1. The ovules are anatropus and bitegmic. Both the integuments take part in the formation of the micropyle which is somewhat zig-zag.

2. The archesporial cell is hypodermal in origin. Two megaspore mother cells have been noted in many preperations. A normal linear tetrad of megaspores is produced of which the lowermost one becomes functional and produces a seven-nucleate embryo-sac of the normal angiospermous type. The antipodals are ephemeral. The development of the embryo is very much delayed. Endosperm development is of the nuclear type.

3. The floss develops from the cells of the epidermal layer of the inner carpellary wall of the boll. All the cells, however, do not develop. The

'non-fibre' cells as also some of the fibre cells divide anticlinally during the early stages of the development of the fibre. The growth of the latter seems to be arrested after division. The mature fibres are uninucleate and have an unconstricted base.

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